A Hexavalent Human Rotavirus–Bovine Rotavirus (UK) Reassortant Vaccine Designed for Use in Developing Countries and Delivered in a Schedule with the Potential to Eliminate the Risk of Intussusception

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There is an urgent need for a rotavirus vaccine, because up to 592,000 infants and young children <5 years old die each year from rotavirus diarrhea, predominantly in the developing countries. We have developed a tetravalent human-bovine rotavirus (UK) reassortant vaccine with VP7 (G) specificity for serotypes 1, 2, 3, and 4, which has been shown to be safe, immunogenic, and effective in preventing severe rotavirus diarrhea. However, because of the emergence of VP7 (G) serotype 9 as an epidemiologically important serotype and the importance of VP7 (G) serotype 8 in focal areas, we are planning to add human-bovine (UK) reassortants with G8 and G9 specificity to the tetravalent vaccine, thereby formulating a “designed” hexavalent vaccine for universal use. In addition, we propose that the vaccine be administered orally in a 2-dose schedule, with the first dose given at 0–4 weeks of age and the second dose given at 4–8 weeks of age, when infants are relatively refractory to developing intussusception, thereby avoiding the age period when naturally occurring intussusception is most prevalent (i.e., ages 3–4 months through age 9 months). In this way, there may be the potential to eliminate or at least significantly decrease the risk of intussusception associated with rotavirus vaccination.

In both developed and developing countries, rotaviruses have consistently been shown to be the single most important cause of severe diarrhea in infants and young children. The consequences of rotavirus diarrhea are staggering, because the disease accounts for up to 592,000 deaths annually among children <5 years old, predominantly in developing countries [1]. It has recently been estimated that 1 in 200 children in developing countries will die of rotavirus diarrhea [2]. In the United States, it was estimated that, among children <5 years old, rotavirus infections are responsible for 2,730,000 episodes of diarrheal illness, 410,000 visits to a physician, 160,000 emergency department visits, 50,000 hospitalizations, and 20 deaths annually [3]. Thus, the need for a rotavirus vaccine in both developed and developing countries has received national and international endorsement.

HISTORICAL PERSPECTIVE: TETRAVALENT RHESUS ROTAVIRUS (RRV)-BASED VACCINE

We developed an oral, live, attenuated tetravalent rotavirus vaccine, with the goal of inducing an immunological response that mimicked the response induced by natural rotavirus infection, especially with regard to induction of immunity at local intestinal sites [4]. This tetravalent vaccine was formulated to protect against...
the 4 epidemiologically important rotavirus serotypes, numbered 1–4. Although the relative importance of homotypic immunity, compared with heterotypic immunity, had not been established with certainty, it appeared from epidemiological, clinical, animal, and laboratory observations that serotype-specific immunity was a major correlate of protection against rotavirus illness [5, 6]. The vaccine comprised representatives of 4 serotypes: RRV, which is a VP7 serotype 3 strain (i.e., the Jennerian approach), and 3 human rotavirus (HRV)–RRV reassortants, each of which possesses 10 RRV genes and a single HRV gene that encodes VP7, a major outer capsid protein that is responsible for serotype 1, 2, or 4 specificity (i.e., the modified Jennerian approach) [4, 7, 8]. Extensive clinical studies demonstrated the safety, immunogenicity, and efficacy of the candidate vaccine, especially against severe diarrhea (up to 91% efficacy) [9–13]. The vaccine protected against VP7 serotypes 1, 3, and 4 but could not be assessed for VP7 serotype 2 protection because of a paucity of such circulating strains in any of the major field trials [9–13]. In addition, in comparative trials of vaccine efficacy with a monovalent and a tetravalent vaccine, when the infecting serotype was heterotypic to that of the monovalent vaccine but homotypic to a component of the tetravalent vaccine, the tetravalent vaccine induced a higher degree of protection overall than did a monovalent vaccine [9, 12, 13].

The US Advisory Committee on Immunization Practices (ACIP) recommended routine administration of the tetravalent vaccine to infants at 2, 4, and 6 months of age [14]. Subsequently, in August 1998, the US Food and Drug Administration granted a Biologics License for the vaccine (RotaShield) to Wyeth–Ayerst [14]. However, in July 1999, after >1 million doses of the vaccine had been administered, the US Centers for Disease Control and Prevention (CDC) recommended suspending its further use pending additional studies, because of a link between vaccination and intussusception, especially during the first 2 weeks after administration of the first dose [15, 16]. Approximately 3 months later, after reviewing additional data, the ACIP withdrew its recommendation for use of the vaccine [16, 17].

This decision has continued to generate intense discussion and controversy in the scientific community because of continuing disagreements about the actual magnitude of the risk of intussusception associated with use of the vaccine and because of related risk/benefit issues [18–27]. These debates are fueled both by the realization that, on a daily basis, up to ~1600 infants and young children worldwide die of a disease [1] that might be prevented if RotaShield were available for use in these settings and by the reality that developing countries will not use a vaccine that has been withdrawn for safety reasons in the United States [18]. Data from the CDC indicated initially that the excess risk of intussusception occurring after administration of RotaShield was as great as 1.8, on the basis of a case-control study (i.e., up to an 80% excess in the number of cases, compared with the background risk, which corresponds to 1 excess case/2500 vaccine recipients) [16, 19, 23]. On the basis of these estimates, the CDC projected that, in a full national vaccination program in the United States, there would be up to 1600 excess cases of intussusception, compared with the background estimate of ~2000 cases/year [16, 19, 23]. However, the relative risk values have undergone considerable downward revisions [28, 29], including the following: (1) an estimated consensus value of 1:10,000 excess cases [30]; (2) population-based hospital discharge studies showing 1:32,000–1:302,000 excess cases among 43–210-day-old infants [23, 31]; and (3) data showing no increase in the number of hospitalizations related to intussusception among infants <1 year old, likely because of a compensatory decrease [23, 31].

More recently, the age at vaccination was shown to be an important factor in the development of intussusception, because vaccine recipients who were >90 days old when the first dose was administered experienced a disproportionately greater number of cases (81%) than did vaccine recipients who were <90 days old, with no cases occurring in infants vaccinated at <60 days of age during the 2 weeks after administration of the first dose in the case-control study conducted by the CDC (figure 1) [32] (L.S., unpublished data). According to analysis of data from the CDC National Immunization Survey, in the 19 states included in the case-control study, infants >90 days old received 38% of all first doses, and infants <60 days old (~70,000 infants) received 16% of all first doses [32]. Thus,

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Age distribution of 43 cases of intussusception that occurred during the 2 weeks after administration of the first dose of RotaShield, in a case-control study conducted by the Centers for Disease Control and Prevention. Data are from Simonsen et al. [32] and L.S. (unpublished data).
“catch-up” vaccination of older infants (i.e., the first dose was given to infants older than the ideally recommended age of 2 months [14]) was responsible for a substantial and disproportionate number of vaccine-associated intussusception cases observed in this study. This observation and its implications are discussed in further detail elsewhere in this issue of The Journal of Infectious Diseases [32].

The US National Institutes of Health (NIH) is embarking on a program of further development of RotaShield as a major public health initiative to prevent the large number of deaths due to rotavirus diarrheal illnesses in infants and young children. In this regard, the NIH Office of Technology Transfer has recently granted an exclusive license for commercialization of RotaShield to BIOVIRx [33]. BIOVIRx is seeking sites to manufacture RotaShield for further evaluation.

**PROGRESS IN THE DEVELOPMENT OF A SECOND-GENERATION TETRAVALENT BOVINE ROTAVIRUS (BRV [UK])–BASED VACCINE**

Concurrent with the development of the HRV-RRV reassortants described above, our laboratory also developed single-gene–substitution HRV–BRV (UK) reassortants comprising 10 genes from the BRV (UK) strain and a gene that encodes VP7, the major outer capsid protein for each of the HRV serotype 1, 2, 3, or 4 strains [7, 8]. We placed our major emphasis on the BRV-based vaccine, which became the first licensed rotavirus vaccine, RotaShield, in the United States.

However, during this period, we also envisioned that our second-generation vaccine would be the BRV-based vaccine, because studies of BRV strain Nebraska calf diarrhea virus (NCDV) strain vaccine had demonstrated that NCDV induced febrile reactions significantly less often than did the RRV strain vaccine [34]. For example, in a direct comparison of febrile responses (rectal temperature, ≥38°C or 100.4°F) after vaccination with monovalent RRV or NCDV, RRV induced a febrile response significantly more often than did NCDV (64% vs. 17%) [34]. We considered this finding to be an advantage for the BRV strain. In addition, because self-limited febrile episodes were also observed with the tetravalent formulaiton of the RRV-based vaccine [9–13], we continued to pursue the BRV (UK)–based tetravalent vaccine actively as the second-generation vaccine.

To achieve the goal of introducing the BRV (UK)–based reassortant vaccine as our second-generation vaccine, we performed phase 1, 2, and 3 clinical studies, and, as described later in the present article, we also generated new candidate strains representing emerging serotypes that could be used in countries where such rotavirus strains were prevalent. These studies have been performed in stepwise fashion, as summarized below:

1. The safety and immunogenicity of each of the 4 monovalent HRV–BRV (UK) reassortant strains, with VP7 specificity for serotype 1, 2, 3, or 4, were evaluated sequentially in adults (1 dose), children (1 dose), and infants (1 or 2 doses) [35]. Each component demonstrated satisfactory attenuation, safety, infectivity, and immunogenicity in the target population of infants 1.5–5.9 months of age.

2. The safety and immunogenicity of the 4 serotypes combined into a tetravalent formulation of the HRV–BRV (UK) reassortant vaccine, with VP7 specificity for serotypes 1, 2, 3, and 4, were evaluated in stepwise fashion in adults (1 dose), children (1 dose), and infants (3 doses) [36]. The tetravalent formulation demonstrated satisfactory attenuation, safety, infectivity, and immunogenicity in the target population of infants 1.5–2.5 months of age. In addition, when given concurrently, the tetravalent formulation did not inhibit antibody responses to diphtheria–tetanus toxoid–pertussis, *Haemophilus influenzae* type b, hepatitis B virus, or oral poliovirus vaccine.

3. The tetravalent HRV–BRV (UK) reassortant vaccine was evaluated for safety, immunogenicity, and efficacy in a field trial performed in Finland using 2 sequential doses, one administered at age ∼2 months and the other administered at age ∼4 months, for ∼170 vaccine recipients and ∼85 control subjects (T.V., unpublished data). It was shown to be safe, and, in contrast to the tetravalent RRV-based vaccine, which was being evaluated in Finland concurrently in a study of approximately the same size, it did not induce febrile episodes at a frequency significantly greater than that noted for the placebo group. The tetravalent HRV–BRV (UK) vaccine induced >80% protection against severe rotavirus diarrhea, an efficacy level comparable to that observed with the tetravalent RRV-based vaccine (T.V., unpublished data).

**DEVELOPMENT OF CANDIDATE STRAINS REPRESENTING ADDITIONAL SEROTYPES FOR ADDITION TO THE TETRAVALENT VACCINE**

We have also developed various single-gene–substitution HRV–BRV (UK) reassortants with a gene of HRV origin encoding VP7 specificity for serotype 5, 8, or 9 or specificity for VP4 1A or 1B in a background of 10 UK genes [37–39]. Similarly, a single-gene–substitution BRV–BRV (UK) reassortant with a gene of BRV origin encoding VP7 with specificity for serotype 10 in a background of 10 UK genes has been developed [37]. The availability of such reassortants affords the opportunity to formulate “designer vaccines” for specific areas of the world, to protect against emerging or unique strains in focal areas of the world. The emergence of unique strains in various parts of the world, as well as the distribution of VP7 1–4 serotypes (also designated as G [glycoprotein]) is shown in figure 2 [40]. For example, G9 strains are now known to be commonly occurring serotypes in numerous developing countries in Asia and Africa [40]. In
addition, in a recent study conducted in Australia, G9 strains were found to be the most frequently occurring serotype [41]. Moreover, G8 strains have also emerged as important strains in various parts of Africa [40]. G5 strains, which were common in Brazil in the 1990s, have decreased in prominence, whereas G10 strains have maintained a low prevalence [40]. Although the VP4 1A serotype (also designated as P [protease sensitive]) is detected in conjunction with various G serotypes, the inconsistency of the association in certain G strains [40] indicates to us that inclusion of prevalent G serotypes would be more practical and effective for inclusion in a multivalent vaccine.

### SECOND-GENERATION HEXAVALENT BRV (UK) VACCINE DESIGNED FOR DEVELOPING COUNTRIES

We propose that our second-generation BRV-based vaccine be formulated for developing countries and that it include not only the standard G1–G4 strains but, also, the G9 and G8 strains, thus comprising a hexavalent vaccine for a broader degree of protection. The composition of such a vaccine is shown in figure 3. The effect of adding 2 additional serotypes to the tetravalent formulation needs to be evaluated with regard to (1) interference among the strains that might influence immunogenicity and (2) the increased cost of manufacturing a hexavalent vaccine.

### A REVISED SCHEDULE FOR ADMINISTRATION OF THE BRV-BASED VACCINE THAT MAY ELIMINATE THE RISK OF EXCESS CASES OF INTUSSUSCEPTION FOLLOWING VACCINATION

It is known that naturally occurring intussusception is relatively rare during the first 2 months of life [42] and that it characteristically peaks at age ∼4–9 months, similar to the pattern observed over the course of ∼7 years by a California health care maintenance organization (figure 4) [43]. A World Health
Figure 3. Human rotavirus (HRV)–bovine rotavirus reassortant hexavalent vaccine with VP7 specificities for serotypes 1, 2, 3, 4, 8, and 9.
The median peak incidence of intussusception occurs at 3–8 months [44]. In view of the recent findings in the United States that, among infants ≥90 days old, a disproportionate number of cases of intussusception occurred in association with catch-up vaccination and that, among infants <60 days old, no cases occurred within 2 weeks of receiving the first dose (figure 1) [32], we propose administration of the hexavalent BRV-based vaccine in a 2-dose regimen, with the first dose administered at 0–4 weeks of age and the second dose administered at 4–8 weeks of age, with a minimum of 3 weeks between the first and second doses. There would be no catch-up vaccinations after 8 weeks of age (i.e., no first or second doses given beyond 8 weeks of age), to avoid vaccination during the highly vulnerable period. In this way, the peak period of vulnerability for developing intussusception under natural conditions (i.e., ∼4–9 months of age) would be avoided, and the occurrence of vaccine-induced intussusception after administration of either the first or second dose might be eliminated. Because of a suggestion of “delayed” intussusception risk associated with later doses, if the first dose is given at an early age [32], we propose avoiding a third dose, because it would have to be administered during the age window of high background intussusception risk.

Will a 2-dose schedule beginning in the first month of life induce satisfactory protection against severe rotavirus diarrhea? This question can be answered only by trials of immunogenicity and/or efficacy. However, evidence from various studies appears to be encouraging: (1) naturally occurring, subclinical rotavirus infection during the neonatal period induced protection against severe rotavirus diarrhea in Australia [45]; (2) 87% of infants studied in Mexico from birth to age 2 years were protected against moderate-to-severe diarrhea after 1 naturally occurring rotavirus infection [46]; (3) neonatal rotavirus vaccination with RIT 4237 BRV (NCDV) vaccine modified the severity of rotavirus gastroenteritis in Finland [47]; and (4) a dose of tetravalent RRV-based vaccine administered during the neonatal period protected against the occurrence of fever associated with receipt of the second vaccine dose at 2 months of age [48] (T.V., unpublished data).

Finally, with this 2-dose schedule (i.e., the first dose administered at 0–4 weeks and the second dose administered at age 4–8 weeks), the risk of postvaccination intussusception may be eliminated, and a further benefit may follow—that is, the vaccine may prevent intussusception due to infection with wild-type rotavirus [23, 49]. However, only large-scale, postlicensure, phase 4 studies will determine whether this schedule will eliminate or significantly reduce the risk of vaccine-associated intussusception. Although studies of the association of wild-type rotavirus infection with intussusception have yielded variable results [43, 44, 50, 51], recent intestinal ultrasound studies showed that wild-type rotavirus induced a significantly greater number of lymph node aggregates and significantly greater thickening of the distal ileum—pathologic alterations that may be a prelude to intussusception—than observed in control infants evaluated by use of coded examinations [52]. There is no assurance that, when 1 million doses of other rotavirus vaccines are given, they will not be linked to rare cases of intussusception [53], especially when given in catch-up situations. Thus, the suggested schedule has the potential to eliminate, or at least significantly decrease, the risk of intussusception linked to rotavirus vaccination, with vaccination beginning in the neonatal period [53] and not extending beyond 8 weeks of age [32].

**CONCLUSION**

We have presented a revised schedule of vaccination for our BRV-based vaccine, which has the potential to eliminate the risk of intussusception because the vaccine would be administered during a period when infants are relatively refractory to the development of intussusception (i.e., at 0–8 weeks of age). In addition, we have proposed the use of a hexavalent BRV (UK)–based vaccine in developing countries, to cover not only the standard serotypes G1–G4 but, also, the emerging serotypes G8 and G9. A similar schedule and formulation may facilitate the reintroduction of RotaShield, because serotype G8 and G9 reassortants are also available for this vaccine [37]. RotaShield has the advantage that >1 million doses have been given to ~600,000 infants [2, 16, 54], whereas, the BRV (UK)–based vaccine has undergone only limited clinical trials. However, the BRV (UK)–based vaccine has the advantage of being associated with significantly fewer febrile responses. It would be of great importance and benefit if rotavirus vaccines could be used safely during infancy, especially
in developing countries, where the consequences of rotavirus infection are so devastating.

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References


