

Review Article

Rotavirus: Approaches to Vaccination

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Abstract: Rotavirus infection is the most common cause of severe diarrhea disease in infants and young children worldwide and continues to have a major global impact on childhood morbidity and mortality. Vaccination is the only control measure likely to have a significant impact on the incidence of severe dehydrating rotavirus disease. In 1999, a highly efficacious rotavirus vaccine licensed in the United States, Rota Shield, was withdrawn from the market after 14 months because of its drawbacks. Two new live, oral, attenuated rotavirus vaccines were licensed in 2006: the pentavalent bovine-human reassortant vaccine (Rota Teq) and the monovalent human rotavirus vaccine (Rotarix). Both vaccines have demonstrated very good safety and efficacy profiles in large clinical trials in western industrialized countries and in Latin America. Careful surveillance has not revealed any increased risk of intussusception in the vaccinated groups with either vaccine. The new rotavirus vaccines are now introduced for routine use in a number of industrialized and developing countries. These new safe and effective rotavirus vaccines offer the best hope of reducing the toll of acute rotavirus gastroenteritis in both developed and developing countries.

Keywords: Rotavirus, diarrhea, vaccination, intussusception and gastroenteritis

INTRODUCTION

Rotavirus gets its name from the fact that, under a microscope, the virus resembles a wheel. And you could say, like you might say about a wheel, rotavirus goes round and round. This nasty, potentially lethal bug causes severe acute gastroenteritis with diarrhea and vomiting primarily in infants and young children. Fortunately, there are two safe rotavirus vaccines that can protect children from this disease.

Rotaviruses are the most common cause of severe diarrhoeal disease in young children throughout the world. Worldwide, diarrheal diseases are a leading cause of pediatric morbidity and mortality, with 1.5 billion episodes and 1.5--2.5 million deaths estimated to occur annually among children aged <5 years [1,2]. 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 [3]. Rarely, involvement of extraintestinal sites, including the respiratory tract, liver, kidney, lymph nodes, and central nervous system, has been reported [4]. Annually in India, rotavirus diarrhoea causes an estimated 122,000-153,000 deaths, 457,000-884,000 hospitalizations, and 2 million outpatient visits in children <5 years of age. India spends Rs 2.0-3.4 billion (US\$ 41-72 million) annually in medical costs to treat rotavirus diarrhoea. Fortunately there are two safe

rotavirus vaccines that can protect children from this disease.

The use of specific interventions against rotavirus, such as newly available vaccines, would help prevent much of this large disease and economic burden [5]. In 2006, the results of pivotal clinical trials of two new rotavirus vaccines — RotaTeq (Merck) and Rotarix (GlaxoSmithKline) — were published, and high efficacy (85 to 98%) against severe rotavirus diarrhea was reported for both products. Perhaps even more important, neither vaccine was associated with intussusception, an adverse effect that had led to the withdrawal of another rotavirus vaccine— RotaShield, made by Wyeth–Lederle — from the U.S. market in 1999. The rapid resurgence of rotavirus vaccines after the abrupt and devastating setback associated with the withdrawal of RotaShield was remarkable, reflecting the commitment of the public health community and the vaccine industry to preventing this most common cause of severe diarrhea in children [6]. The WHO currently has regional surveillance systems in place to document rates of disease from rotavirus. It is critical to maintain such surveillance systems to track the safety and effectiveness of the vaccine and shifts in serotype distribution [7].

ROTAVIRUS VACCINES

Improvements in sanitation and hygiene have had a great impact on reducing diarrheal disease and deaths due to bacterial and parasitic agents that are spread primarily through contaminated food or water, but these improvements have had a lesser impact on infection with rotavirus, which is most commonly

spread from person to person [8]. Instead, early studies that followed cohorts of children in their first 2–3 years of life identified epidemiologic features of rotavirus that indicated the disease might best be controlled through vaccination. Specifically, these studies demonstrated that children previously infected with rotavirus were protected against subsequent disease. Protection was greatest against moderate-to-severe disease and the level of protection increased with each new infection [9]. These data implied that an attenuated rotavirus vaccine that mimics natural infection could provide protection against disease and that multiple vaccine doses would probably be required to confer optimal protection.

HISTORY

In 1998, a rotavirus vaccine (RotaShield, Wyeth) was licensed for use in the United States. Clinical trials in the United States, Finland, and Venezuela had found it to be 80 to 100% effective at preventing severe diarrhea caused by rotavirus A, and researchers had detected no statistically significant serious adverse effects. The manufacturer of the vaccine, however, withdrew it from the market in 1999, after it was discovered that the vaccine may have contributed to an increased risk for intussusceptions, or bowel obstruction, in one of every 12,000 vaccinated infants. The experience provoked debate about the relative risks and benefits of a rotavirus vaccine.[10]

In 2006, two vaccines against Rotavirus A infection were shown to be safe and effective in children: Rotarix by GlaxoSmithKline and RotaTeq by Merck [11]. Both are taken orally and contain disabled live virus.

ROTAVIRUS DISEASE BURDEN

Rotavirus infects nearly every child by the age of 3-5 years. The median age of a primary rotavirus infection is younger in developing countries, ranging from 6 to 9 months (80% occur among infants <1 year old). Developing countries often exhibit one or more periods of more intense rotavirus circulation against a background of year-round rotavirus transmission and a great diversity of rotavirus strains. In contrast, the median age of primary infection is older in developed countries, ranging from 9 to 15 months (65% occur among infants <1 year old) caused by 4 to 5 common rotavirus strains. Despite nearly universal rotavirus infections early in life, these differences between developing and developed countries, as well as differences in health care access, childhood co-infections and co-morbidities, drive substantial differences in disease burden.

Every year, rotavirus gastroenteritis is estimated to cause approximately 527,000 (475,000-580,000) deaths globally among children <5 years old [12]. Most of these deaths occur in developing countries and 90% of the rotavirus-associated fatalities occur in

Africa and Asia alone [13]. Globally, >2 million children are hospitalized each year for rotavirus infections. In a recent report of sentinel hospital-based rotavirus surveillance from 35 nations representing each of the six WHO regions between 2001 and 2008, an average of 40% (range= 34%-45%) of hospitalizations for diarrhea among children < 5 years old were attributable to rotavirus infection [14]. Using standardized surveillance techniques[15], the 2001-2008 report indicated a median rotavirus hospitalization detection rate of 34% in the Americas, 40% in both Europe and the Eastern Mediterranean, 41% in Africa, and 45% in South East Asia and the Western Pacific. These proportions are far greater than two previous estimates of rotavirus-attributable hospitalizations in international settings. A previous median rotavirus detection rate of 22% was reported in one review of studies that had been published during 1986-1999 [16], and another review from 1990-2004 reported that a median of 29% of diarrheal hospitalizations were caused by rotavirus [17].

First rotavirus infections are most likely to result in moderate-severe cases of rotavirus gastroenteritis but subsequent infections are progressively milder. Velazquez et al.[18] found that the adjusted efficacy of a child's first natural rotavirus infection in protecting against subsequent natural rotavirus-associated diarrhea was 77%. This protection increased to 83% after two natural infections and to 92% after three natural infections. A study compared rotavirus-infected neonates with uninfected neonates who were followed for three years. A similar proportion of neonatally infected and uninfected infants had rotavirus infections during the follow-up period. Symptoms among those neonatally infected, however, were less frequent and less severe (P=0.003) leading to the conclusion that neonatal rotavirus infection protects against clinically severe disease during reinfection [19]. A similar finding was reported among a sample of Indian neonates infected nosocomially, most of whom had asymptomatic infections, resulting in a protective effect against rotavirus gastroenteritis lasting throughout the 2 year follow-up period, with protection concentrated in the first year of life[20].

THE RATIONALE FOR VACCINATION AS THE PRIMARY PREVENTIVE MEASURE

Control measures such as clean water initiatives and improvements to personal hygiene have led to dramatic declines in bacterial and parasitic gastroenteritis infections across the world, but rates of rotavirus infection and illness among children in industrialized and less-developed countries remain similar. Hygienic measures are unlikely to lead to corresponding declines in rotavirus burden [21]. The ubiquity of the virus, its minute infectious dose, and its environmental stability greatly facilitate rotavirus transmission. Despite the availability of oral rehydration solution, rotavirus continues to cause

disease morbidity and mortality, even in areas having improved sanitation. Since first infections have been shown to induce strong immunity against severe rotavirus re-infections, and since vaccination mimics such first infections without causing illness, vaccines have been identified as the optimal strategy to decrease the burden associated with severe and fatal rotavirus diarrhea. Clinical and population-based studies have demonstrated that the new rotavirus vaccines are safe and efficacious in preventing severe rotavirus-associated diarrhea and mortality and reduce the impact upon public health resources.

VIROLOGY

Types of rotavirus

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. There are seven species of rotavirus, referred to as A, B, C, D, E, F and G. Humans are primarily infected by species A, B and C, most commonly by species A. All seven species cause disease in other animals.

Within rotavirus A there are different strains, called serotypes. As with influenza virus, a dual classification system is used, which is based on two structural proteins on the surface of the virion. The glycoprotein VP7 defines G-types and the protease-sensitive protein VP4 defines P-types. Strains are generally designated by their G serotype specificities (e.g., serotypes G1 to G4 and G9), and the P-type is indicated by a number and a letter for the P-serotype and by a number in square brackets for the corresponding P-genotype. (P-serotypes are difficult to characterize; therefore, molecular methods based on sequence analysis are often used to define the corresponding P-genotype instead. These genotypes correlate well with known P-serotypes). Because the two genes that determine G-types and P-types can be passed on separately to offspring, various combinations occur in any one strain. The Wa strain is classified in full as G1P1A [22].

Structure

The genome of rotavirus consists of 11 unique double helix molecules of RNA which are 18,555 nucleoside base-pairs in total. Each helix or segment is a gene, numbered 1 to 11 by decreasing size. Each gene codes for one protein, except genes 9 and 11, which each code for two [23]. The RNA is surrounded by a three-layered icosahedral protein capsid. Viral particles are up to 76.5 nm in diameter and are not enveloped.

Proteins

There are six viral proteins (VPs) that form the virus particle (virion). These structural proteins are called VP1, VP2, VP3, VP4, VP6 and VP7. In addition to the VPs, there are six nonstructural proteins (NSPs) that are only produced in cells infected by rotavirus.

These are called NSP1, NSP2, NSP3, NSP4, NSP5 and NSP6. At least six of the twelve proteins encoded by the rotavirus genome bind RNA [24]. The role of these proteins play in rotavirus replication is not entirely understood; their functions are thought to be related to RNA synthesis and packaging in the virion, mRNA transport to the site of genome replication, and mRNA translation and regulation of gene expression [25].

Structural proteins

VP1 is located in the core of the virus particle and is an RNA polymerase enzyme [26]. In an infected cell this enzyme produces mRNA transcripts for the synthesis of viral proteins and produces copies of the rotavirus genome RNA segments for newly produced virus particles. VP2 forms the core layer of the virion and binds the RNA genome. VP3 is part of the inner core of the virion and is an enzyme called guanylyl transferase. This is a capping enzyme that catalyses the formation of the 5' cap in the post-transcriptional modification of mRNA [27]. The cap stabilises viral mRNA by protecting it from nucleic acid degrading enzymes called nucleases. VP4 is on the surface of the virion that protrudes as a spike. It binds to molecules on the surface of cells called receptors and drives the entry of the virus into the cell. VP4 has to be modified by a protease enzyme (found in the gut) into VP5* and VP8* before the virus is infectious. It determines how virulent the virus is and it determines the P-type of the virus. VP6 forms the bulk of the capsid. It is highly antigenic and can be used to identify rotavirus species [28]. This protein is used in laboratory tests for rotavirus A infections [29]. VP7 is a glycoprotein that forms the outer surface of the virion. Apart from its structural functions, it determines the G-type of the strain and, along with VP4, is involved in immunity to infection.

Nonstructural viral proteins

NSP1, the product of gene 5, is a nonstructural RNA-binding protein [30]. NSP2 is an RNA-binding protein that accumulates in cytoplasmic inclusions (viroplasm) and is required for genome replication. NSP3 is bound to viral mRNAs in infected cells and it is responsible for the shutdown of cellular protein synthesis. NSP4 is a viral enterotoxin to induce diarrhoea and was the first viral enterotoxin discovered.

NSP5 is encoded by genome segment 11 of rotavirus A and in virus-infected cells NSP5 accumulates in the viroplasm [31]. NSP6 is a nucleic acid binding protein, and is encoded by gene 11 from an out of phase open reading frame [32]. 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.

IMMUNOLOGY & 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 [33]. 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 [34]. 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. Exposure of neonates (asymptotically) to rotavirus is associated with a reduced likelihood of their developing severe rotavirus diarrhea later in infancy [35]. 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. 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 [36]. 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. 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. VP6 is the immunodominant antigen in the antibody response to human rotavirus infection [37]. 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. A high level of serum IgA antibody correlates with clinical protection against rotavirus gastroenteritis. Neutralizing antibodies against VP7 and VP4 antigens clearly play a role in protection after natural rotavirus infection, 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 [38]. 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. More recently, human rotaviruses attenuated by passage in cell culture have been developed and tested [39]. Finally, rotaviruses recovered from asymptomatic human neonates, which may be naturally less virulent, are being developed as oral vaccine candidates [40].

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) has been developed by Merck Research Co. This vaccine contains five live reassortant rotaviruses [41]. 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 [42]. 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 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)[43]. 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 [44]. 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 [45]. 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 [46].

VACCINES BASED ON HUMAN ROTAVIRUS

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

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 [35]. 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) 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. 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. A subset of 20,000 infants in this large trial was monitored for efficacy [47]. 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 [48]. 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. The first dose should be given at 6 weeks and should be given no later than at the age of 12 weeks. The interval between two

doses should be at least 4 weeks. The two dose schedule should be completed by age 16 weeks and no later than by 24 weeks of age. If the infant spits out or regurgitates the entire vaccine dose may be repeated at the same visit. [49]

CONCLUSION

Despite this potential for rotavirus vaccines to substantially reduce the risk of death from diarrhea, there are considerable challenges to implementing their use in the poorest countries of the world. The rotavirus vaccines are currently recommended for administration during a narrow window: the first dose between 6 and 15 weeks of age, and the third dose no later than 32 weeks of age [50]. This recommendation is a serious impediment to the widespread use of rotavirus vaccines, especially in countries with the highest child mortality, which tend to have the lowest vaccine coverage and the lowest rate of on-time immunization. The current cost of the rotavirus vaccines per dose in the United States is far beyond the means of most middle-income countries and the poorest countries. Fortunately, cofinancing is available, at least for the short term, from GAVI (formerly known as the Global Alliance for Vaccines and Immunization) — resulting in a cost of 15 to 30 cents per dose, depending on the economic status of the country. The remainder of the cost is absorbed by GAVI; therefore, it is imperative that the global donor community continues to support programs such as GAVI to ensure that the poorest children have access to these new lifesaving vaccines. The vaccines should be introduced immediately in areas with high mortality from rotavirus infection, and their introduction should be used to energize diarrhea-control programs and improve coverage for all the proven interventions for diarrhea. It is time to act to combat the 1.8 million unnecessary deaths from diarrhea that continue to occur each year [51].

REFERENCES

1. Kosek M, Bern C, Guerrant RL. The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. *Bull World Health Organ* 2003; 81(3):197--204.
2. Parashar U, Hummelman E, Bresee J, Miller M, Glass R. Global illness and deaths caused by rotavirus disease in children. *Emerg Infect Dis* 2003; 9:565--72.
3. Blutt SE, Kirkwood CD, Parreno V, Warfield KL, Ciarlet M. Rotavirus antigenaemia and viraemia: a common event? *Lancet* 2003; 362 :1445-1449.
4. Lynch M, Lee B, Azimi P, Gentsch J, Glaser C, Gilliam S, et al. Rotavirus and central nervous system symptoms: cause or contaminant? Case reports and review. *Clin. Infect. Dis.* 2001; 33:932-938.
5. Tate JE, Chitambar S, Esposito DH, Sarkar R, Gladstone B, Ramani S, et al. Centers for Disease Control and Prevention, Atlanta, GA, USA. *Vaccine* 2009; Nov 20 ; 27 Suppl 5:18-24.
6. Umesh D. Parashar MB, M.P.H BS, and Roger I, Glass MD .Rotavirus Vaccines — Early Success, Remaining Questions. *Global health* 2009; 360(11):1063-1065.
7. Global networks for surveillance of rotavirus gastroenteritis, 2001-2008. *Wkly Epidemiol Rec* 2008; 83: 421-425.
8. Rodriguez WJ, Kim HW, Brandt CD et al. Longitudinal study of rotavirus infection and gastroenteritis in families served by a pediatric medical practice: clinical and epidemiologic observations. *Pediatr. Infect. Dis. J.* 6(2), 170–176 (1987).
9. Velazquez FR, Matson DO, Calva JJ et al. Rotavirus infections in infants as protection against subsequent infections. *N. Engl. J. Med.* 335(14), 1022–1028 (1996).
10. Bines J (2006). "Intussusception and rotavirus vaccines". *Vaccine* 24 (18): 3772–6.
11. O’Ryan M (2007). "Rotarix (RIX4414): an oral human rotavirus vaccine". *Expert review of vaccines* 6 (1): 11–9.
12. World Health Organization. Rotavirus vaccines. *Weekly Epidemiological Record* 2007; 32:285-296.
13. Parashar UD, Hummelman EG, Bresee JS, Miller MA, Glass RI. Global illness and deaths caused by rotavirus disease in children. *Emerg Infect Dis* 2003;9:565-72.
14. Glass RI, Bresee JS, Turcios R, Fischer TK, Parashar UD, Steele AD. Rotavirus vaccines: targeting the developing world. *J Infect Dis* 2005;192: S160-6.
15. Centers for Disease Control and Prevention. Rotavirus surveillance – Worldwide, 2001-2008. *MMWR* 2008; 57: 1255-8.
16. World Health Organization. Summary report on meeting to standardize new vaccines surveillance data to be collected, shared and reported. October 6-7, 2008. <http://www.who.int/nuvi/Summary%20Report.pdf>
17. Parashar UD, Burton A, Lanata C, et al. World Health Organization estimates of the global mortality from rotavirus in children in the year 2004. *J Infect Dis* 2009; in press.
18. Velazquez FR, Matson DO, Calva JJ, et al. Rotavirus infection in infants as protection against subsequent infections. *N Engl J Med* 1996; 335:1022-1028.
19. Bishop RF, Barnes GL, Cipriani E, Lund LS. Clinical immunity after neonatal rotavirus infection. A prospective longitudinal study in young children. *N Engl J Med* 1983. 309:72-6.
20. Bhan MK, Lew JF, Sazawal S, Das BK, Gentsch JR, Glass RI. Protection conferred by neonatal rotavirus infection against subsequent rotavirus diarrhea. *J Infect Dis* 1993; 168:282-287.
21. Mrukowicz J, Szajewska H, Vesikari T. Options for the prevention of rotavirus disease other than

- vaccination. *J Pediatr Gastroenterol Nut* 2008; 46 Suppl 2:S32-37.
22. Dennehy PH. "Rotavirus vaccines: an overview". *Clin. Microbiol. Rev.* 2008; 21 (1): 198–208.
 23. Desselberger U, Wolleswinkel-van den Bosch J, Mrukowicz J, Rodrigo C, Giaquinto C, Vesikari T, Rotavirus types in Europe and their significance for vaccination. *Pediatr. Infect. Dis. J.* 2006; 25 (1 Suppl.): S30–41. [10] Chan WK, Penaranda ME, Crawford SE, Estes MK. Two glycoproteins are produced from the rotavirus neutralization gene. *Virology* 1986;151 (2): 243–52.
 24. Patton JT .Structure and function of the rotavirus RNA-binding proteins. *J. Gen. Virology* 1995; 76 (Pt 11): 2633–44.
 25. Patton JT. Rotavirus RNA replication and gene expression. *Novartis Found. Symp.* 2001; 238: 64–77; discussion 77–81.
 26. Vásquez-del Carpió R, Morales JL, Barro M, Ricardo A, Spencer E .Bioinformatic prediction of polymerase elements in the rotavirus VP1 protein. *Biol. Res.* 2006; 39 (4): 649–59.
 27. Fresco LD, Buratowski S. Active site of the mRNA-capping enzyme guanylyltransferase from *Saccharomyces cerevisiae*: similarity to the nucleotidyl attachment motif of DNA and RNA ligases . *Proc. Natl. Acad. Sci. U.S.A.* 1994 ; 91 (14): 6624–8.
 28. Bishop RF. Natural history of human rotavirus infection. *Arch. Virol. Suppl.* 1996; 12: 119–28.
 29. Beards GM, Campbell AD, Cottrell NR, Peiris JS, Rees N, Sanders RC, Enzyme-linked immunosorbent assays based on polyclonal and monoclonal antibodies for rotavirus detection *J. Clin. Microbiol.* 19 (2): 248–54.
 30. Hua J, Mansell EA, Patton JT. Comparative analysis of the rotavirus NS53 gene : conservation of basic and cysteine-rich regions in the protein and possible stem-loop structures in the RNA. *Virology* 1993 ; 196 (1): 372–8.
 31. Afrikanova I, Miozzo MC, Giambiagi S, Burrone O .Phosphorylation generates different forms of rotavirus NSP5. *J. Gen. Virol* 1996; 77 (Pt 9): 2059–65.
 32. Mohan KV, Atreya CD. Nucleotide sequence analysis of rotavirus gene 11 from two tissue culture-adapted ATCC strains, RRV and Wa. *Virus Genes* 2001;23 (3): 321–9.
 33. Chrystie IL, Totterde BM, Banatvala JE. Asymptomatic endemic rotavirus infections in the newborn. *Lancet* 1978; 312:1176-1178.
 34. Fischer T K, Valentiner-Branth P, Steinsland H , Perch M , Santos G, Aaby P, et al. Protective immunity after natural rotavirus infection: a community cohort study of newborn children in Guinea-Bissau, west Africa. *J. Infect. Dis.* 2002; 186:593-597.
 35. Bishop RF, Barnes GL, Cipriani E, and Lund JS. Clinical immunity after neonatal rotavirus infection. A prospective longitudinal study in young children. *N. Engl. J. Med.* 1983; 309:72-76.
 36. Newman R D, Grupp-Phelan J , Shay DK, Davis RL. Perinatal risk factors for infant hospitalization with viral gastroenteritis. *Pediatrics* 1999; 103:E3.
 37. Velazquez FR, Matson DO, Calva JJ, Guerrero L, Morrow AL, Carter-Campbell S, et al. Rotavirus infections in infants as protection against subsequent infections. *N. Engl. J. Med.* 1996; 335:1022-1028.
 38. Ward RL. Possible mechanisms of protection elicited by candidate rotavirus vaccines as determined with the adult mouse model. *Viral Immunol.* 2003;16:17-24.
 39. Svensson L, Sheshberadaran H, Vesikari T, Norrby E, Wadell G. Immune response to rotavirus polypeptides after vaccination with heterologous rotavirus vaccines (RIT 4237, RRV-1). *J. Gen. Virol.* 1987; 68:1993-1999.
 40. Franco MA, Feng N and Greenberg HB. Molecular determinants of immunity and pathogenicity of rotavirus infection in the mouse model. *J. Infect. Dis.* 1996;174(Suppl. 1):S47-S50.
 41. Velazquez FR, Matson DO, Guerrero ML, Shults J, Calva JJ, Morrow AL, Pickering, Ruiz-Palacios GM. Serum antibody as a marker of protection against natural rotavirus infection and disease. *J. Infect. Dis.* 2000; 182:1602-1609.
 42. Chiba S, Yokoyama T, Nakata S, Morita Y, Urasawa T, Taniguchi K et al.. Protective effect of naturally acquired homotypic and heterotypic rotavirus antibodies. *Lancet* 1986; ii:417-421.
 43. Franco MA, Angel J, and Greenberg HB. Immunity and correlates of protection for rotavirus vaccines. *Vaccine* 2006; 24:2718-2731.
 44. Hoshino Y and Kapikian AZ. Rotavirus vaccine development for the prevention of severe diarrhea in infants and young children. *Trends Microbiol* 1994; 2:242-249.
 45. Bernstein D I, Sack D A, Rothstein E, Reisinger K, Smit V E , O'Sullivan D, Spriggs DR and Ward R L. Efficacy of live, attenuated, human rotavirus vaccine 89-12 in infants: a randomised placebo-controlled trial. *Lancet* 1999; 354:287-290.
 46. Barnes G L, Lund JS, Mitchell SV, De Bruyn L, Piggford L, Smith AL, et al. Early phase II trial of human rotavirus vaccine candidate RV3. *Vaccine* 2002; 20:2950-2956.
 47. Glass RI, Bhan MK, Ray P, Bahl R., Parashar UD, Greenberg H, et al. Development of candidate rotavirus vaccines derived from neonatal strains in India. *J. Infect. Dis.* 2005;192(Suppl. 1):S30-S35.
 48. Vesikari T, Matson DO, Dennehy P, Van Damme P, Santosham M, Rodriguez Z, et al. Safety and efficacy of a pentavalent human-bovine (WC3) reassortant rotavirus vaccine. *N. Engl. J. Med.* 2006; 354:23-33.
 49. Parashar UD, Alexander JP, and Glass RI. Prevention of rotavirus gastroenteritis among infants and children. Recommendations of the Advisory

Committee on Immunization Practices (ACIP).
MMWR Recommend. Rep 2006; 55:1-13.

50. Centers for Disease Control and Prevention.
Postmarketing monitoring of intussusception after

RotaTeq vaccination—United States, February 1,
2006-February 15, 2007. MMWR Morb. Mortal.
Wkly. Rep. 2007; 56:218-222.