Rotavirus

LÚCIA HELENA DE OLIVEIRA
AND MARIA TEREZA DA COSTA OLIVEIRA

SABIN
VACCINE INSTITUTE

Rotavirus

Lúcia Helena De Oliveira
New Vaccines Advisor, Immunization Unit/FGL, Pan American Health Organization/World Health Organization, Washington, DC

Maria Tereza da Costa Oliveira
Consultant, Immunization Unit/FGL, Pan American Health Organization/World Health Organization. Washington, DC

Introduction

Rotavirus is the leading cause of severe, dehydrating diarrhea among children aged <5 years globally.[1] Since 2006, two rotavirus vaccines are available and were introduced into 93 countries worldwide by January 2018.[2] Several countries that have implemented routine childhood vaccination against rotavirus have documented a tremendous impact on severe diarrhea and rotavirus disease requiring hospitalization. Additionally, some countries in the Region of the Americas, including Mexico, Brazil, and Panama, have documented substantial decreases of 22%–50% in diarrhea mortality among children <5 years of age following vaccine introduction.[1]

Etiological Agent

Rotaviruses belong to the *Reoviridae* family, *Rotavirus* genus. The viral particles were first identified by Bishop et al. by direct visualization on electron microscopy in 1973, in a duodenal mucosa biopsy[3,4] and stools[4,5] from children with acute diarrhea. Viral particles have typical morphology, similar to a cartwheel (based on which researchers suggested the "rotavirus" denomination).[6-8] They are 80nm to 100nm in diameter, icosahedral structure, three-layer protein capsid and no viral envelope, which increases their resistance to soluble lipids and other adverse environmental conditions. They are very stable and may remain viable in the environment during weeks or months absent disinfestation.[4,9]

The viral genome contains 11 double-stranded RNA segments, contained in a nuclear capsid. The viral genome segments encode 6 structural proteins: VP1, VP2, VP3, VP4, VP6, VP7; and 6 non-structural proteins: NSP1, NSP2, NSP3, NSP4, NSP5, and NSP6, with the exception of segment 11 which codes for two proteins (NSP5 and NSP6).[4,8]

The intermediate capsid comprises the VP6 protein, encoded by segment 6. VP6 is the most abundant protein in the virion and the basis for classification of rotaviruses into serotype groups, ranging from A to H, and for serologic identification through ELISA test.[4,10] Groups A, B, C, and H have been described in human-beings and animals, while the others (D-G) have been found only in animals (mammals and birds).[4,11] Group A has been identified as the most important one for public health since it is a significant cause globally of severe diarrhea in young children.[4,11,12]

Every serogroup may be classified into several genotypes as determined by the VP4 (P protein) and VP7 (G protein) proteins located in the external capsid. These proteins have multiple epitopes inducing the synthesis of neutralizing antibodies and, therefore, they may impact on the efficacy of the rotavirus vaccines.[4,8,9] Twenty-seven genotypes for the G protein, and 37 genotypes for the P protein have been described, with the G1P[8], G2P[4], G3P[8], G4P[8], and

G9P[8] combinations accounting for the highest number of cases. Most of the genotypes circulate concomitantly during a season and facilitate viral reassortment, which combined with genome mutations and rearrangements, are considered to be the main mechanisms for the evolution of the rotavirus genetic diversity.[13-15]

Pathogenesis

The rotavirus transmission model is not well known even though the disease is communicated through personal contact (oral-fecal transmission), contaminated fomites or air sprays.[16-18] Viral replication takes place in the small intestinal villi, advancing from the proximal to the distal areas.[9,17]

There are two principal mechanisms for diarrhea to occur: osmotic imbalance and secretion. Rotavirus infection produces an extensive necrosis of the enteric epithelium, which impairs intestinal absorption of sodium, glucose, water, lactose, and sucrose, thus inducing isotonic diarrhea.[9,11,19] This is followed by reactive crypt hyperplasia with increased intestinal secretion, which also contributes to the severity of diarrhea.[4,19]

The secretory mechanism is produced from the release of the NSP4 protein acting as a viral enterotoxin in addition to its role in viral replication and intracellular morphogenesis. This enterotoxic effect is produced on uninfected cells when interfering with the Ca2+ ion metabolism, increasing its intracellular concentration and altering the electrolytic homeostasis, which accounts for the acute diarrhea observed even before histopathological changes in the epithelium and even absent very extensive damage.[4,11,19]

The enteric nervous system may also be involved in rotavirus-induced diarrhea, since the substances blocking this system mitigate diarrheal symptoms. Likewise, although viremia is apparently frequent, systemic disease is rare, suggestive of rotavirus spread to other organs concurrently with systemic disease caused by other organisms.[4,19] In sum, rotavirus-induced diarrhea is a complex mechanism involving poor absorption, hypersecretion, and alteration of intestinal permeability and motility. The severity of the disease is dependent on the characteristics of the virus and the host.[4,11,19]

Rotavirus infection triggers an intestinal and systemic local immune response, despite being an infection that mainly affects the intestinal mucosa.[20] The primary rotavirus infection produces specific homotypic humoral immunity, typically not permanent. After the first natural infection, 38% of infected children have been observed to have protection for an ensuing infection, 77% were protected against diarrhea and 87% against acute diarrhea. The ensuing infections produce homotypic and heterotypic immunity, offer greater protection and are usually less severe than the primary infection.[9,21-23]

Incubation and Communicable Period

Transmission is mainly fecal-oral.[9,24,25] Incubation is relatively short, usually less than 48 hours and the disease has a sudden onset.[9,11,19] Transmission is high since it requires a low infectious inoculum and the number of virus particles excreted in diarrhea is very high, and may reach 10^11 viral particles/mL feces before and after onset of symptoms, during the acute phase of the disease.[19,24] Virus excretion starts before the onset of symptoms and continues even upon conclusion of diarrhea; based on studies with immunoassays it varies from 4 to 29 days, with a 7-day median, while molecular tests (PCR) have detected virus excretion in a 4–57 day range with a 10 day median.[4] The viral particle is very resistant to the environment, and it may persist for up to 10 days on dry surfaces and 4 hours on human hands.[25]

Clinical Characteristics

The rotavirus infection is more frequent and more severe in children 3 to 36 months of age.[26] The clinical manifestations vary depending on whether it is the first infection or a reinfection. The infection may be asymptomatic, induce self-limiting watery diarrhea or produce severe diarrhea accompanied by fever and vomiting. Other symptoms are quite rare, such as central nervous system impairment, hepatitis, and chronic infections. The first infection, after the first three months of life, is usually the most severe.[9,16,23,26] Diarrhea usually lasts 3 to 8 days with 10 to 20 daily episodes. Fever and vomiting are more frequent at the outset. Fever is usually low but up to a third of the children may have temperatures higher than 38.5°C–39.0°C with the risk of suffering from febrile seizures. Vomits occur in 80% to 90% of the cases of severe diarrhea; they are usually severe and last less than a day.[9,16,23,27] Rotavirus-induced diarrhea is usually more associated with dehydration and hospitalization than diarrhea induced by other agents.[4,27] Infection in immunocompromised individuals due to bone marrow transplant or another type of transplant may present extended viral excretion, as well as severe symptoms and higher risk of death.[4,9]

Rotavirus-induced diarrhea is clinically similar to diarrhea induced by other agents. Case confirmation requires laboratory tests, including immunoassays such as ELISA or rapid tests from agglutination tests, usually in stool samples.[9,19] Treatment is mainly channeled to rehydration of the patient, by mouth or parenterally, with the recommended addition of zinc since it has been proven to reduce the diarrhea duration. For rehydration by mouth, the use of oral rehydration salts with low-osmolarity is recommended.[18,19]

Epidemiology

Rotavirus is globally the leading cause of diarrhea in children aged <5 years, both in developing and in developed countries, which is suggestive of the fact that the infection cannot be prevented by solely improving sanitation services since viruses equally affect different geographic areas, social or ethnic groups.[7,9,11,28] In low income countries, the median age at the primary rotavirus infection ranges from 6 to 9 months (80% occur among infants <1 year old). Whereas in high income countries, although the majority still occur in infancy (65% occur among infants <1 year old), the first episode may occasionally be delayed until the age of 2–5 years.[18,29]

As of April 2016, the World Health Organization (WHO) estimates that globally 215,000 (197,000–233,000) child deaths occurred during 2013 due to rotavirus infection compared to 528,000 (465,000–591,000) in 2000, but it is still the most important cause of diarrhea-related death.[1] About 90% of these fatalities occurred in low-income countries, in particular in Africa and Asia.[18] National estimates of rotavirus attributable deaths among children under five years of age ranged from 47,100 (India) to fewer than 5 deaths (79 countries). Twenty-two percent of all rotavirus deaths under five years of age occurred in India. Four countries (India, Nigeria, Pakistan and the Democratic Republic of the Congo) accounted approximately half (49%) of all rotavirus deaths under age five in 2013. Globally these 215,000 child rotavirus deaths accounted for approximately 3.4% of all child deaths and the cause-specific mortality rate (rotavirus deaths under age five per 100,000 population under age five) was 33.[1]

Before the vaccine was available, it was estimated that 1 out of every 5 children received medical care and that 1 out of every 50 to 70 was hospitalized in the first 5 years of life due to rotavirus-induced infection.[30] This accounted for a total of 114 million episodes of gastroenteritis that required home treatment, 24 million clinic

visits and 2.4 million hospitalizations in children <5 years globally.[31] Likewise, the disease has been determined to have a seasonal pattern occurring more frequently during winter months.[9]

Globally, prevalent genotypes are G1P[8], G2P[4], G3P[8], and G9P[8]. Genotype distribution may be different for various seasons or years.[32] G1P[8] is the most frequently isolated genotype subject to seasonal and regional variation.[32]

Before the introduction of the rotavirus vaccine in Latin America and the Caribbean, there were 15,000 deaths, 75,000 hospitalizations, and 10 million cases of rotavirus-caused diarrhea annually. Data from epidemiological surveillance in 11 countries and territories showed that the median percentage of positive specimens for rotavirus in hospitalized cases was 31.5% and 39.0% in 2006 and 2007, respectively.[33] A meta-analysis conducted with studies published between 1990 and 2009 showed that the percentage of positive specimens for rotavirus in hospitalized patients was 29.7%.[12] In 2011, epidemiological surveillance showed a median percentage of positive specimens in 15 countries of 19.0%, after the introduction of the vaccine in several countries of the region.[34]

Rotavirus-induced mortality estimated for the 2005–2007 period for 10 countries in the Latin American region showed that 1 out of every 2,874 children <5 years died due to rotavirus, for a total of 3,492 deaths and a 34.8 per 100,000 rate among children <5 years, which was consistent with estimates published by the World Health Organization (WHO) in 2004.[28,33] The mortality rate estimated in a meta-analysis with data from 22 countries for the 1977–2009 period was 88.2 (79.3–97.1) per 100,000 children <5 years.[12]

As observed in other countries, a seasonal pattern has been observed, with a higher number of cases and percentage of positive specimens in the months from November to March in Northern-Hemisphere countries while in Southern-Hemisphere countries cases occur with higher frequency is in the months of May to September.[33]

The more frequently circulating genotypes in Latin America and the Caribbean for the 2005–2007 period were G1P[8] (32.0%), G9P[8] (20.9%), and G2P[4] (18.3%), based on information from the surveillance system.[50] Similar information published in a 2011 meta-analysis for the period prior to 2010 showed G1P[8], 17.9% (12.2%–24.4%), G2P[4], 9.1% (4.9%–14.5%), and G9P[8] 8.8% (4.1%–15.0%) as the most frequent genotypes.[12]

Availability of Vaccines

Efforts to find a vaccine against rotavirus started in the 1970's, driven by the recognition, in 1979, of rotavirus as a significant cause of infant morbidity and mortality by WHO.[11,35] However, it was not until February 1998 that the first tetravalent vaccine was licensed in the United States (G1-G4) in a three-dose schedule (2, 4, and 6 months) to prevent rotavirus-induced diarrhea.[35] However, the vaccine was interrupted in July 1999 by recommendation of the U.S. Centers for Disease Control and Prevention (CDC) due to the detection of intussusception after vaccination. There were no new cases after administration of the vaccine was suspended.[36] Years later, two new vaccines have been licensed, with an excellent safety profile.[18]

Rotavirus Specie A (RVA) Vaccine

The two vaccines currently available in the international market against RVA are: 1) the live, attenuated monovalent (G1P[8]) vaccine Rotarix™ (GlaxoSmithKline), and 2) the reassortant pentavalent (G1-4P[8]) vaccine Rotateq™ (Merck). Two other vaccines (by the Lanzhou Institute of Biomedical Products, China) and Rotavin-M1 (manufactured by Polyvac, Vietnam) are not available in the international market.[18]

The Monovalent Vaccine (RV1)

The RV1 is a live single-strain vaccine of genotype G1P[8] derived from a human rotavirus strain. This strain has undergone 43 passages of tissue culture and the resulting attenuated vaccine strain, RIX4414, is propagated in Vero cells. The vaccine is administered orally in a two-dose schedule. The first vaccine should be administered to infants at 6–14 weeks of age, and the second dose should be administered at a 4-week interval. According to the manufacturer, the second dose should be administered prior to 24 weeks of age.[18,37]

The first clinical studies were conducted in Finland and enrolled 63,225 children. They showed a 42% efficacy (29.0%–53.0%) for the reduction of hospitalizations due to all-cause diarrhea. In Latin America and Asia, the efficacy was 70.0% to 85.0% for RVA-induced diarrhea and 85.0% to 93.0% for acute RVA diarrhea. The vaccine proved to be safe with no excessive risk of causing intussusception in vaccinated children. It was first licensed in Mexico in 2004 and then in other countries of Europe and the Americas.[9,37,38]

The Pentavalent Vaccine (RV5)

The RV5 vaccine was developed from an attenuated bovine virus WC3, of genotype GXPY. This genotype was reassorted at the laboratory and the genotypes G1-G4 and P[8] from the human strains were added. Four strains express one of the VP7 proteins G1-G4 from a human strain and the VP4 protein P7[5] from a bovine strain. The fifth strain expresses a VP4 protein P1A[8] from a human strain and the VP7 protein G6 from a bovine strain. They are propagated in Vero cells using culture techniques.[18,39]

The vaccine is administered orally in a three-dose schedule. Based on the manufacturer's recommendation, the first dose should be administered at 6–12 weeks of age and subsequent doses should be administered at intervals of 4–10 weeks. The first three doses should be administered by 32 weeks of age.[18,40]

This vaccine was approved in clinical trials conducted in more than 70,000 children, mainly in the United States and Finland, although studies were also conducted in South America, Europe, and Asia. The clinical studies showed 94.5% (92.2%–96.6%) efficacy in the reduction of hospitalizations and emergency department visits related to RVA-induced diarrhea. Other studies had efficacies of 74.0% (66.8%–79.9%) for all-cause diarrhea due to RVA and 98.0% (88.3%–100.0%) for acute diarrhea due to RVA. The risk of intussusception was similar among the vaccinated and unvaccinated children.[40] It was first licensed in the United States in February 2006.[41]

Both vaccines, RV1 y RV5, have high efficacy and an excellent safety profile,[42] and they were prequalified by WHO in January 2007 and August 2008, respectively.[43,44]

Vaccine Recommendations

WHO recommends the administration of either vaccine against RVA starting at 6 weeks of age, before 24 months of age, concomitantly with the other vaccines in the national vaccination schedules. The goal is for a greater number of children, in particular in low-income countries, to have access to vaccination. An incremental mortality study demonstrated that an additional 21% to 28% death could be prevented by moving from a restricted vaccination schedule to an unrestricted schedule for date of initiation. RV1 should be administered in a 2-dose schedule, with a 4 week interval between doses. RV5 should be administered at the time of DTP1, DTP2, and DTP3, with an interval of 4 weeks between doses.[18]

In October 2012, the Pan American Health Organization (PAHO) though its Technical Advisory Group (TAG), also recommended starting vaccination after the dates established in the WHO recommendations in children who live in areas difficult to access with a high mortality risk. In all cases, the vaccine should be administered as early as possible.[45] Countries introducing the RVA vaccine should monitor for the occurrence of intussusception to guarantee the safety of the vaccine in the immunization programs, and the baseline incidence of this disease should be estimated prior to vaccine introduction.[18,46]

Rotavirus Vaccine Introduction in Latin America and the Caribbean

Six countries in the region (Brazil, El Salvador, Mexico, Nicaragua, Panama, and Venezuela) introduced the rotavirus vaccine into their national immunization schedules in 2006, the same year the vaccine was licensed. For the first time in history, developing countries introduced a vaccine at the same time as developed countries.[47] However, the introduction took place before the implementation of rotavirus surveillance, contrary to the recommendation by PAHO/WHO.[34,48] Other factors must have impacted the decision, such as local publications on rotavirus.[49]

As of December 2016, 21 countries and one territory in Latin America and the Caribbean had included a rotavirus vaccine, where 96% of the target population is estimated to live. The most widely-used vaccine is the monovalent, which is not used in only Mexico and the Cayman Islands.

Impact of the Rotavirus Vaccine in Latin America

Vaccine Effectiveness

Both vaccines have demonstrated high levels of effectiveness in the studies published. A meta-analysis published in 2012 reviewed this data.[51] This study included 29 clinical trials (101,671 participants) to study the RV1 and 12 clinical trials (84,592 participants) to study the RV5. The results for the RV1 study are shown in Table 1 and the results for the RV5 study are shown in Table 2.

Table 1. RV1 Effectiveness for Diarrhea Prevention

Age/Scope	Countries With a Low Mortality Rate	Countries With a High Mortality Rate
Infants <1 Year	RV1 prevents 86% cases of acute diarrhea (RR=0.14, 95% CI: 0.07–0.26) and 40% episodes of all-type diarrhea (RR=0.60, 95% CI: 0.50–0.72).	RV1 prevents 63% of acute diarrhea cases (RR=0.37, 95% CI: 0.18–0.75) and 34% episodes of all-type diarrhea (RR=0.66, 95% CI: 0.44–0.98).
Children Up to 2 Years of Age	RV1 prevents 85% cases of acute diarrhea (RR=0.15, 95% IC: 0.12–0.20) and 37% episodes of all-type diarrhea (RR=0.63, 95% CI: 0.56-0.71).	RV1 prevents 42% cases of acute diarrhea (RR=0.58, 95% CI: 0.42–0.79) and 18% episodes of all-type diarrhea (RR=0.82; 95% CI: 0.71–0.95).

Table 2. RV5 Effectiveness for Diarrhea Prevention

Age/Scope	Countries With a Low Mortality Rate	Countries With a High Mortality Rate
Infants <1 Year	RV5 prevents 87% cases of acute diarrhea (RR=0.13, 95% CI: 0.04–0.45) and 72% episodes of all-type diarrhea (RR=0.28, 95% CI: 0.16–0.48).	RV5 prevents 57% of acute diarrhea cases (RR=0.43, 95% CI: 0.29–0.62). Data was insufficient to assess episodes of all-type diarrhea.
Children Up to 2 Years of Age	RV5 prevents 82% cases of acute diarrhea (RR=0.18, 95% CI: 0.07–0.50) and 96% episodes of all-type diarrhea (RR=0.04, 95% CI: 0.00–0.70).	RV1 prevents 41% cases of acute diarrhea (RR=0.59, 95% CI: 0.43–0.82) and 15% episodes of all-type diarrhea (RR=0.85; 95% CI: 0.75–0.98).

The study groups had no differences as to adverse events or frequency of intussusception in particular. Efficacy was similar for both vaccines and it was higher for acute diarrhea, in children <1 year of age and in countries with low-mortality rate.[51]

Effectiveness in LAC

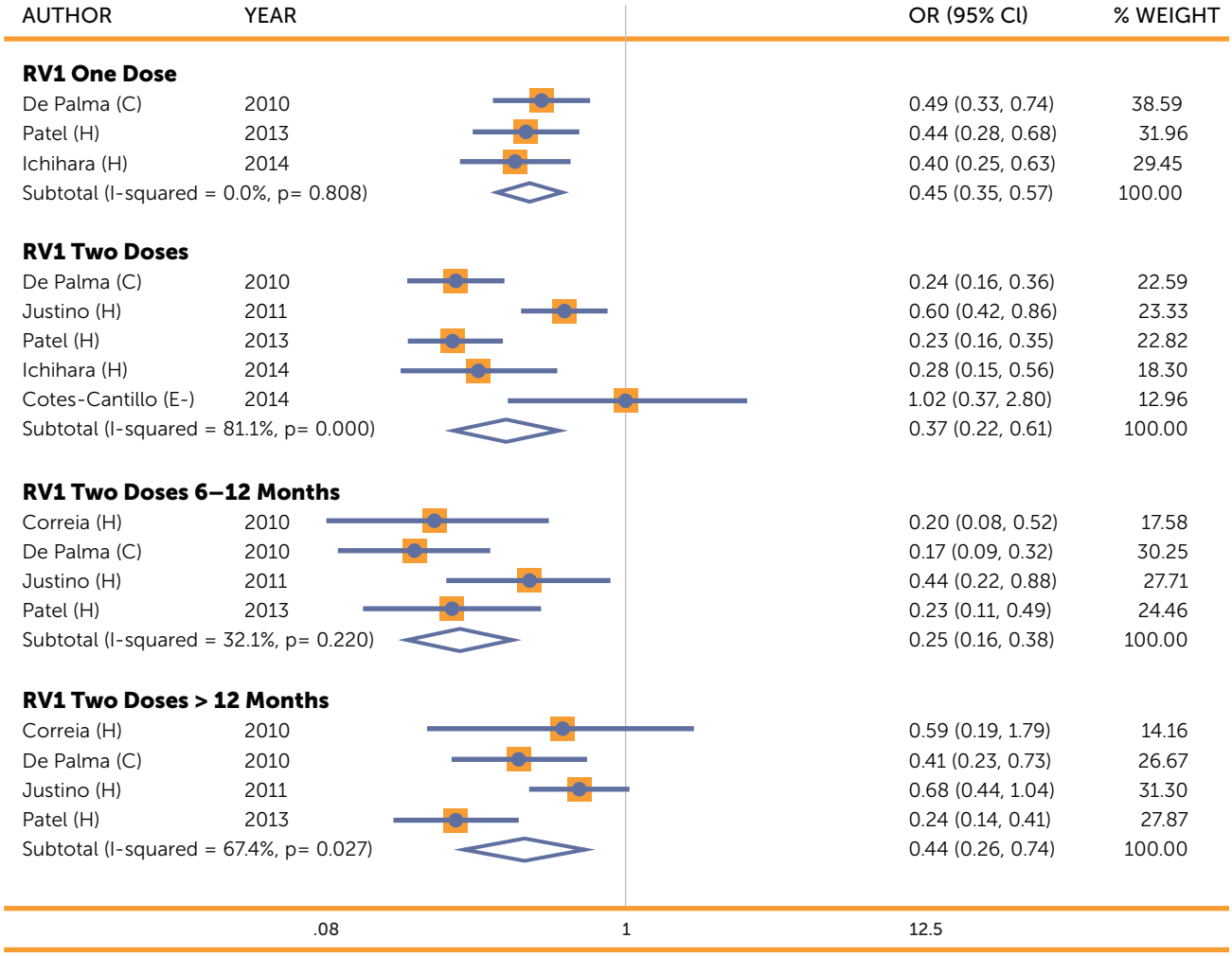
A meta-analysis[52] published in 2015 of studies with data from the region demonstrated both vaccines are effective in preventing hospitalizations due to rotavirus-induced diarrhea. This research included 8 case-control studies, with a total of 6,265 cases and 21,448 controls. The estimates were based on different control types, which led to the identification of different levels of effectiveness.

The results found for RV1 were:

- Effectiveness of two doses to prevent RVA-induced hospitalizations ranged between 63.5% (95% CI: 39.2%–78.0%) and 72.2% (95% CI: 60.9%–80.2%).
- Effectiveness of two doses in children <1 year to prevent hospitalizations ranged between 75.4% (95% CI: 64.6%–82.9%) and 81.8% (95% CI: 72.3%–88.1%).
- Effectiveness of two doses in children >1 year to prevent hospitalizations ranged between 56.5% (95% CI: 26.2%–74.3%) and 66.4% (95% CI: 54.1%–75.5%).

Figure 1 shows the odds ratio (OR) results in each RV1 study selected for the meta-analysis.

Figure 1. Effectiveness of the Monovalent Rotavirus Vaccine, Based on Dose and Age



NOTE: Weights are from random effects analysis **RV1: Monovalent Vaccine** **C: Community Control** **H: Hospital Control** **E-: EIA Test Negative**

Source: De Oliveira et al., 2015.[52]

RV5 results were:

- Effectiveness to prevent diarrhea with a Vesikari score >11 in infants at 6–11 months of age ranged between 76.1% (95% IC: 57.6%–86.6%) and 88.8% (95% CI: 78.3%–94.3%).
- The effectiveness to prevent hospitalized diarrhea cases caused by G2P[4] was 63.5% (95% CI: 29.4%–82.6%).

In sum, RVA vaccines offered consistent protection against diarrhea-induced hospitalization in LAC. The effectiveness was significant for hospital and community controls but higher in the latter group. Likewise, effectiveness was higher in children <12 months of age.

Finally, PAHO estimates that as of 2013 RVA vaccination has prevented between 6,903 and 8,621 deaths in children <5 years of age.

Conclusion

To conclude, it is important to consider the following:

- RVA vaccination should be administered and completed within the schedule as soon as possible. RV1 requires two separate doses with at least a 4-week interval and RV5 requires three doses, with a 4-week interval also.
- Efficacy and effectiveness studies have demonstrated the significant impact this vaccine has on morbidity caused by diarrhea in developed and developing countries.
- Epidemiological surveillance is important to monitor disease trends, study genotype distribution, and characterize RVA epidemiological profile.
- For effectiveness studies, it is important to analyze the impact for various genotypes.
- Studies to analyze trends suggest a significant reduction of morbidity and mortality, in children <5 due to the RVA vaccine.

References

1. Tate JE, Burton AH, Boschi-Pinto C, Parashar UD, World Health Organization–Coordinated Global Rotavirus Surveillance Network, Agocs M, Serhan F, de Oliveira L, Mwenda JM, Mihigo R, Ranjan Wijesinghe P. Global, regional, and national estimates of rotavirus mortality in children< 5 years of age, 2000–2013. *Clinical Infectious Diseases*. 2016 Apr 7;62(suppl_2):S96-105.

2. WHO/IVB Database, as of 26 January 2018. Map production Immunization Vaccines and Biologicals (IVB),World Health Organization [cited 2018 May, 1st]; Available from: http://www.who.int/immunization/monitoring_surveillance/data/en/.

3. Bishop RF, Barnes GL, Cipriani E, Lund JS. Clinical immunity after neonatal rotavirus infection. A prospective longitudinal study in young children. *N Engl J Med*. 1983;309(2):72-6.

4. Estes M, Kapikian A. Rotaviruses. En: Fields B, Knipe D, Howley P, editors. *Fields Virology*. 5ta ed. Philadelphia: Wolters Kluwer Health/ Lippincott Williams & Wilkins, 2007. p. 1917-74.

5. Flewett TH, Bryden AS, H. D. Virus particles in gastroenteritis. *Lancet*. 1973;2(7844):1497.

6. Flewett TH WG. The rotaviruses. *Arch Virol*. 1978;57(1):1-23.

7. Parashar UD, Bresee JS, Gentsch JR, Glass RI. Rotavirus. *Emerg Infect Dis*. 1998; 4(4):561-70.

8. Patton JT. Rotavirus diversity and evolution in the post-vaccine world. *Discov Med*. 2012;13(68):85-97.

9. Centers for Disease Control and Prevention. Epidemiology and Prevention of Vaccine-Preventable Diseases. Hamborsky J, Kroger A, Wolfe S, editors. 13 ed. Washington D.C.: Public Health Foundation, 2015.

10. Molinari B, Otonel R, Alfieri A, Alfieri A. Species H Rotavirus Detected in Piglets with Diarrhea, Brazil, 2012. *Emerging Infectious Diseases* 2014;20(6).

11. Schael IP. Vacuna de rotavirus: una agenda global para su desarrollo y aplicación universal. Bogotá, Colombia: Editorial Medica Panamericana; 2012.

12. Linhares AC, Stupka JA, Ciapponi A, Bardach AE, Glujovsky D, Aruj PK, et al. Burden and typing of rotavirus group A in Latin America and the Caribbean: systematic review and meta-analysis. *Rev Med Virol*. 2011;21(2):89-109.

13. de Sá AC. Deteccao e caracterizacao genotipica de rotavirus da especie A e norovirus em amostras fecais humanas de Fortaleza, Ceara.: Fundação Oswaldo Cruz. Instituto Oswaldo Cruz; 2012.

14. Iturriza-Gomara M, Isherwood B, Desselberger U, Gray J. Reassortment in vivo: driving force for diversity of human rotavirus strains isolated in the United Kingdom between 1995 and 1999. *J Virol*. 2001;75(8):3696-705.

15. Payne DC, Szilagyi PG, Staat MA, Edwards KM, Gentsch JR, Weinberg GA, et al. Secular variation in United States rotavirus disease rates and serotypes: implications for assessing the rotavirus vaccination program. *Pediatr Infect Dis J*. 2009;28(11):948-53.

16. Clark FH, Offit PA, Parashar UD. Rotavirus vaccine. In: Plotkin SA, Orestein W, Offit PA, editors. Vaccines. 6th ed. London, UK: Elsevier Saunders; 2013. p. 669-87.

17. Pérez-Vargas J, Isa P, López S, Arias CF. Rotavirus vaccine: early introduction in Latin America- risk and benefits. *Arch Med Res*. 2006;37(1):1-10.

18. World Health Organization. Rotavirus vaccines: WHO position paper. *Wkly Epidemiol Rec*. 2013 88:49-64;88:49-64.

19. Desselberger U, Manktelow E, Li W, Cheung W, Iturriza-Gomara M, Gray J. Rotaviruses and rotavirus vaccines. *Br Med Bull*. 2009;90(1):37-51.

20. Ward R. Mechanisms of protection against rotavirus in human and mice. *J Infect Dis*. [supplement]. 1996;174:51-8.

21. Bányai K, László B, Duque J, Steele AD, Nelson EAS, Gentsch JR, et al. Systematic review of regional and temporal trends in global rotavirus strain diversity in the pre rotavirus vaccine era: Insights for understanding the impact of rotavirus vaccination programs. *Vaccine*. 2012;30:A122-A30.

22. Velazquez FR, Matson DO, Calva J, Guerrero L, Morrow AL, Carter-Campbell S, et al. Rotaviruses and rotavirus vaccines. *Br Med Bull*. [article]. 2009:37-51.

23. Staat MA AP, Berke T, Roberts N, Bernstein DI, Ward RL ,Pickering LK, Matson DO. Clinical presentations of rotavirus infection among hospitalized children. *Pediatr Infect Dis J*. 2002;21(3):221-7.

24. Richardson S, Grimwood K, Gorrell R, Palombo E, Barnes G, Bishop R. Extended excretion of rotavirus after severe diarrhoea in young children. *Lancet.* 1998;351(9119):1844-8.

25. Ansari SA, Springthorpe VS, Sattar SA. Survival and vehicular spread of human rotaviruses: possible relation to seasonality of outbreaks. *Rev Infect Dis*. 1991;13(3):448-61.

26. Anderson E, Weber S. Rotavirus infection in adults. *Lancet Infect Dis*. 2004;4(2):91-9.

27. Grimwood K CR, Barnes GL, Bishop RF. Patients with enteric adenovirus gastroenteritis admitted to an Australian pediatric teaching hospital from 1981 to 1992. *J Clin Microbio*. 1995;33(1):131-6.

28. Glass RI, Bresee JS, Turcios R, Fischer TK, Parashar UD, Steele AD. Rotavirus vaccines: targeting the developing world. *J Infect Dis*. 2005 Sep 1;192(Suppl 1):S160-6.

29. World-Bank. Country and lending groups 2014 [cited 2014 May, 31st]; Available from: http://data.worldbank.org/about/country-classifications/country-and-lending-groups#Low_income.

30. O'Ryan M, Matson DO. New rotavirus vaccines: Renewed optimism. *J Pediatr*. 2006;149(4):448-51.

31. 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(5):565-72.

32. World Health Organization. Global rotavirus information and surveillance bulletin. Vol. 6: Report from January to December 2011. 2012 [cited 2013 May 16]; Available from: http://www.who.int/immunization/diseases/rotavirus/RV_bulletin_Jan_Dec_2011_FINAL.pdf?ua=1.

33. de Oliveira L, Danovaro-Holliday MC, Andrus JK, de Fillipis AM, Gentsch J, Matus CR, et al. Sentinel hospital surveillance for rotavirus in Latin american and Caribbean countries. *J Infect Dis.* 2009; 200 (Suppl 1):S131-S9.

34. Organización Panamericana de la Salud. Inmunización en las Américas: resumen 2012. . Washington, DC: OPS; 2012 [cited 2014 Jun 16]. Available from: http://new.paho.org/hq/index.php?option=com_docman&task=doc_view&gid=19048&Itemid=.

35. Centers for Disease Control and Prevention. Rotavirus vaccine for the prevention of rotavirus gastroenteritis among children. Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR* Morb Mortal Wkly Rep [serial on the Internet]. 1999; 48(RR-2): Available from: http://www.cdc.gov/mmwr/preview/mmwrhtml/00056669.htm#top.

36. Centers for Disease Control and Prevention. Suspension of rotavirus vaccine after reports of intussusception-United States, 1999. *MMWR* Morb Mortal Wkly Rep. 2004;53(34):786-9.

37. Ruiz-Palacios GM, Perez-Schael I, Velazquez FR, Abate H, Breuer T, Clemens SC, et al. Safety and efficacy of an attenuated vaccine against severe rotavirus gastroenteritis. *N Engl J Med*. 2006 Jan 5;354(1):11-22.

38. Eisenberg S. The case of the pyrogenic platelet product. ONS Connect. 2013 Dec;28(4):45.

39. Hsieh Y, Wu F, Hsiung C, Wu H, Chang K, Huang Y. Comparison of virus shedding after lived attenuated and pentavalentreassortant rotavirus vaccine. *Vaccine*. 2014;32(10):1199-204.

40. 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(1):23-33.

41. Centers for Disease Control and Prevention. Prevention of rotavirus gastroenteritis among Infants and children. Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR* Morb Mortal Wkly Rep [serial on the Internet]. 2006 55: Available from: http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5512a1.htm.

42. Glass RI, Parashar UD, Bresee JS, Turcios R, Fischer TK, Widdowson MA, et al. Rotavirus vaccines: current prospects and future challenges. *Lancet*. 2006;368(9532):323-32.

43. Jiang J, Jiang B, Parashar U, Nguyen T, Bines J, Patel MM. Childhood intussusception: a literature review. *PLoS One*. 2013;8(7):e68482.

44. World Health Organization. Rotavirus vaccine pre-qualification. 2009 [31 de Maio de 2014]; Available from: https://extranet.who.int/gavi/PQ_Web/.

45. Organización Panamericana de la Salud. Marcando el rumbo en inmunización: reunión XX del Grupo Técnico Asesor (GTA) sobre enfermedades prevenibles por vacunación: informe final. Washington DC: OPS; 2012.

46. World Health Organization. Grading of scientific evidence: tables 1-4: does RV1 and RV5 induce protection against rotavirus morbidity and mortality in young children both in low and high mortality settings? 2013 [31 Mai 2014]; Available from: http://www.who.int/immunization/position_papers/rotavirus_grad_rv1_rv5_protection.pdf.

47. de Oliveira L, Danovaro-Holliday MC, Matus CR, Andrus JK. Rotavirus vaccine introduction in the Americas: progress and lessons learned. *Expert Rev Vaccines*. [article]. 2008;7(3):345-58.

48. World Health Organization. Generic protocols: hospital-based surveillance to estimate the burden of rotavirus gastroenteritis in children and a community-based survey on utilization of health care services for gastroenteritis in children. Field test version. Geneva: WHO; 2002 [cited 2014 Jun 16]. Available from: http://www.who.int/immunization/documents/WHO_VB_02.15/en/index.html.

49. de Oliveira LH, Toscano CM, Sanwogou NJ, Ruiz-Matus C, Tambini G, Roses-Periago M, et al. Systematic documentation of new vaccine introduction in selected countries of the Latin American Region. *Vaccine*. 2013 Jul 2;31 Suppl 3:C114-22.

50. Pan American Health Organization. Country reports to PAHO: data and statistics (IM. 2014 [cited 2014 Jun 10]; Available from: www.paho.org/immunization/data.

51. Soares-Weiser K. Rotavirus vaccines schedules: a systematic review of safety and efficacy from randomized controlled trials and observational studies of childhood schedules using RV1 and RV5 vaccines. Washington DC: WHO; 2012; Available from: http://www.who.int/immunization/sage/meetings/2012/april/Soares_K_et_al_SAGE_April_rotavirus.pdf.

52. de Oliveira LH, Camacho LA, Coutinho ES, Ruiz-Matus C, Leite JP. Rotavirus vaccine effectiveness in Latin American and Caribbean countries: A systematic review and meta-analysis. *Vaccine*. 2015;33 Suppl 1:A248-54. doi: 10.1016/j.vaccine.2014.11.060.