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Trends in Rotavirus Vaccine Administration

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Trends in Rotavirus Vaccine Administration

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An abstract of

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In partial fulfillment of the requirements for the degree of

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ABSTRACT

Introduction: Rotavirus vaccine was introduced in Nicaragua in 2006 and was recommended with upper age limits for completing the first dose (by age 105 days) and the final dose (by age 223 days) for the three dose series to minimize age specific risk of intussusception following rotavirus vaccination. Several years after introduction, estimated coverage with rotavirus vaccine was lower compared to coverage for other recommended childhood immunizations.

Methods: This was a retrospective cohort study which analyzed data from a previously conducted vaccine effectiveness study in Nicaragua from 2006 to 2010. It examined trends in adherence to the upper age limit restriction (of 105 and 223 days) for administration of rotavirus vaccine in children under 5 years of age in Nicaragua. Logistic regression was used to estimate an odds ratio (OR) of receiving rotavirus vaccine after the age limit, among children presenting to the clinic after the age limit. Multivariate models were constructed to identify other factors (apart from the age restriction) that are associated with the receipt of rotavirus vaccine after each age limit.

Results: Among children receiving their 1st dose of pentavalent vaccine after the age limit of 105 days, the likelihood of simultaneously receiving rotavirus vaccine was decreased by 90 % (O.R = 0.1 (95%CI: 0.08, 0.16)) compared to children receiving their rotavirus vaccine before the age limit, as shown in Table 4. This pattern for dose 1 remained consistent over time. Among children receiving their 3rd dose of pentavalent vaccine after the age limit of 223 days, the likelihood of simultaneously receiving rotavirus vaccine was decreased by 40% (O.R = 0.6 (95%CI: 0.2, 0.8)) in 2006, 30 % (O.R = 0.7 (95% CI: 0.3, 0.4)) in 2007, 10 % (O.R = 0.9 (95% CI: 0.7, 1.2) in 2008 and (O.R = 3.8 (95%CI: 1.9, 7.2)) in 2009 compared to children receiving pentavalent vaccine before the age limit as shown in Table 4. For doses 1 and 3, among children receiving pentavalent vaccine after the respective age limits, the odds of receiving rotavirus vaccine was higher in other cities when compared to Managua. For dose 3 alone, maternal education was found to be positively associated with receiving rotavirus vaccine after the age limit of 223 days of age, and children diagnosed with pneumonia had a reduced likelihood of receiving this vaccine.

Conclusions: This study found that age restrictions was associated with limited rotavirus coverage. The adherence to such restrictions, especially the third dose, waned over time, suggesting that physicians may have shown increased interest in assuring full rotavirus immunization, as experience with using the vaccine progressed over time.

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Contents

1. INTRODUCTION

1.1 Discovery

Rotavirus was first discovered in 1973 when Ruth Bishop, et al. studied intestinal biopsies of children with acute gastroenteritis (AGE) via electron microscopy (1). Virus particles found in stool were given the name “rotavirus’’ because the particle resembled a wheel (Latin: rota) when visualized in an electron microscope (2). Rotavirus was later observed to be responsible for a substantial proportion of cases of acute gastroenteritis among children below 5 years of age, globally, and are disproportionately represented as a particular cause of moderate to severe gastroenteritis (6).

1.2 Epidemiology

Before vaccine introduction, Rotavirus-related diarrhea was estimated to cause 453,000 deaths annually worldwide in children younger than 5 years (3). It is estimated that more than 90% of these deaths occur in low-income countries in Africa and Asia (4). Rotavirus, the most common cause of severe diarrhea, accounts for 30 % of all diarrheal disease cases and 5% of all deaths in this age group (5). The Global Enteric Multicenter Study (GEMS) across several countries in Africa and south Asia, found that rotavirus was the leading cause of diarrhea when compared with other enteric pathogens. In the first year of life it was reported to have an incidence of 8 cases per 100 child years and 4 cases per 100 child years among children 1 to 2 years of age (6). Rotavirus disease leads

to around 114 million episodes of gastroenteritis requiring only home care, 24 million clinic visits, and 2.4 million hospitalizations (3, 7-11). It is estimated that each child is normally infected at least once by the age of 2 years (12). In temperate climates there are distinct winter and spring seasonal peaks of rotavirus disease. In tropical climates, rotavirus circulates year-round but frequently has peaks during the cool or dry months (13).

1.3 Transmission

The main form of transmission is through the fecal-oral route (contaminated water or contaminated fomites) (14). The viral shedding load is around 10^{10} per gram of feces and fewer than 100 particles are needed to infect new contacts (15). The risk of spreading the disease can be mitigated by adequate hygiene and use of disinfectants which inactivated the virus.

1.4 Clinical disease and treatment

The incubation period for rotavirus is approximately 1 to 3 days with an abrupt onset comprising of fever, vomiting and explosive watery diarrhea (16, 17). Disease usually subsides after 8 days. Complications and death are due to dehydration, electrolyte imbalance and acidosis (17).

Rotavirus infection cannot be reliably diagnosed by clinical presentation and stool examination. Rapid test kits using the principle of Enzyme immunoassay – based and latex agglutination tests are available commercially and Reverse Transcription (RT-PCR) are used to detect viral loads (12, 18). Treatment is largely supportive and includes nonspecific methods, such as rehydration therapy for replacement of body fluids and electrolytes (19).

The high disease burden and lack of antivirals urged the development of three efficacious and safe rotavirus vaccines (20-26). These vaccines are orally administered within the first 6 months of life. Until recent years, vaccines have been available only in developed countries but are now gradually making their way into the National Immunization programs of various developing countries.

These vaccines are: *Rotarix* (GlaxoSmithKline Biologicals, Rixensart, Belgium) and *RotaTeq* (Merck Vaccines, Whitehouse Station, NJ, USA) and *Rotavac* (Bharat Biotech, Hyderabad, India). Rotarix (GSK) is a monovalent live attenuated human rotavirus strain given as two doses, RotaTeq (Merck) is a pentavalent bovine derived rotavirus strain given as three doses and Rotavac (Bharat Biotech) is a live naturally attenuated human rotavirus strain given as three doses (20, 24, 25). These vaccines enter the gut and due to attenuation have a limited number of replication cycles conferring immunity in the form of IgA antibodies. The immunity it develops is robust and lasts for 2 years with some evidence of it waning (20, 24, 27).

In October 2006, Nicaragua introduced the 3-dose Rotateq vaccine series with recommended administration at 2, 4 and 6 months of age. This was recommended to be concurrently given with injected pentavalent vaccine (diphtheria-tetanus-pertussis-hepatitis B-*Haemophilus influenzae* type b [Hib]) and oral Polio vaccine (14).

1.5 Immunization recommendations

As Nicaragua was classified as a low-income country, it was GAVI (The Global Alliance for Vaccines and Immunizations) eligible to introduce rotavirus vaccine into the national immunization program in 2006. Dataset from a rotavirus vaccine effectiveness study conducted in Nicaragua from 2006 to 2010 was analyzed to explore trends in rotavirus vaccine coverage and adherence to the upper age limit restriction.

2. BACKGROUND

2.1 Vaccine effectiveness

Vaccine effectiveness, which is often confused with vaccine efficacy, should be viewed as the 'real world' view of how a vaccine whereas vaccine efficacy quantifies this reduction in controlled settings and may prove to have a higher reduction of disease in a population (28).

The currently licensed vaccines have shown moderate efficacy (50% – 60%) in South Africa, Malawi, Kenya, Ghana, and India. High efficacy (75 – 90 %) was found in America and Finland (20, 24, 27, 29, 30). Effectiveness studies of rotavirus vaccine have also shown highly varying results throughout the world, ranging from 50 – 75% effectiveness in preventing severe rotavirus gastroenteritis (31-36). A study in 4 Latin American countries showed that vaccine effectiveness against rotavirus-related hospitalizations was the lowest (46%) in Nicaragua (37). The vaccine was shown to be least effective in low-income countries, which may be explained by malnutrition, poor sanitation, competing pathogens in those settings. Nonetheless, one study found that the introduction of rotavirus vaccine in Nicaragua led to a reduction in diarrheal episodes (incidence rate ratios: 0.85, 0.71-1.02) (38).

Globally, it is estimated that 2.4 million rotavirus related deaths and more than 82 million disability-adjusted life years (DALYs) in 64 of the 72 GAVI-eligible countries would be prevented as a result of the introduction of rotavirus vaccine from 2007

through 2025. Hence, introduction of rotavirus vaccines into the world's poorest countries is thought to be very cost-effective and estimated to substantially reduce mortality in children (39).

2.2 Risk of intussusception

Rotashield, the first ever licensed rotavirus vaccine was withdrawn from the market in 2000 due to evidence supporting the association with a condition known as intussusception (peak onset 3-4 months of age). This condition is characterized by the telescoping of one part of the small intestine into an adjacent part, leading to bowel obstruction. Complications include bowel ischemia and death, and resolution of the condition often requires surgical intervention in infants. The incidence of intussusception with Rotashield was estimated to be 1 case per every 10,000 vaccinations (26, 40-42). Even though current vaccines have shown a much lower risk, this led the scientific community to consider options to limit the age at which children were administered with the vaccine.

2.3 Upper age limit restrictions

Due to previous concerns about intussusception, in 2006 when newer rotavirus vaccines were introduced, the WHO-SAGE (Strategic Advisory Group of Experts) recommended upper age limits of 15 weeks of age for the first dose and 8 months of age for completion of the final dose in the series (43). In the Americas, the Pan American Health Organization (PAHO) Technical Advisory Group on vaccine-preventable diseases,

recommended compliance with the age restriction of administering rotavirus vaccine. However, later administration (up to one year of age) of rotavirus vaccine was considered to be justified in regions with high mortality due to rotavirus infections (44). These recommendations made rotavirus vaccines the only vaccines in immunization program schedule with an upper age limit for administration (10).

The coverage of rotavirus vaccine is generally lower than that of co-administered vaccines (14, 45-47). Recent literature has shown that the lower coverage rates were attributable to the recommended age restrictions (45, 46). Patel et al, showed that in low income countries, a restricted schedule would prevent 155,800 deaths related to rotavirus while causing 253 intussusception deaths. In contrast, vaccinations without this age restriction would prevent 203,000 deaths related to rotavirus and potentially cause 547 intussusception deaths. Thus, removing the age restriction would increase rotavirus vaccine coverage and result in a benefit: risk ratio of 150 child deaths averted for every one vaccine-related death due to intussusception (48). In 2012, the WHO revised their recommendation to co-administer the rotavirus vaccine along with pentavalent and polio vaccines regardless of age (49).

3. METHODS

3.1 Aims

These are the following research questions.

1. To examine trends in adherence to the upper age limit restriction (of 105 and 223 days) for administration of rotavirus vaccination in children under 5 years of age in Nicaragua in the period after the WHO recommended this age restriction.

2. To identify factors that were associated with the administration of rotavirus vaccine after the age limit.

3.2 Data sources

This is a retrospective cohort design and secondary analysis of a de-identified dataset from a Rotateq vaccine effectiveness case-control study in Nicaragua conducted from 2006 - 2010. The study was a joint collaboration between the Ministry of Health Department, Nicaragua and the Centers for Disease Control and Prevention, (CDC), USA – Division of Viral Diseases. The subjects for this study were the 'control' subjects in the joint study.

3.3 Subject selection and Inclusion criteria

The study was conducted in four regional sites in Nicaragua: Managua, Matagalpa, Masaya and Carazo. Subjects for this study had to be control subjects for the original CDC/Nicaragua study, born within 30 days of the case patients in the original study (age matched), and had to have been age eligible to receive their 1st dose of Rotateq. They were sourced from the case patient's neighborhood or hospital. Neighborhood controls had to live in the same neighborhood as the case patient. Hospital controls had to be admitted to the same hospital, as close to the time of the case patient and may have presented with any condition provided that they were rotavirus test negative.

For the purpose of these analyses, case patients were excluded as they would have had a lower likelihood to be vaccinated with rotavirus and would not serve as an appropriate representation of the population of children in Nicaragua. Hence, data from children who were enrolled as controls was analyzed.

3.4 Exclusion criteria

For this study, children were excluded if they met the following criteria:

1. Unavailable information regarding administration of vaccination from either vaccine cards or medical records.
2. Child did not receive their 1st dose of pentavalent vaccine.

3. Child was age ineligible to receive their 3rd dose of rotavirus vaccine at the time of enrolment.

3.6 Statistical analysis

Descriptive characteristics for continuous variables were presented with their means (SD) or medians (quartiles). Continuous variables were presented as numbers (percentages). The cohort was stratified into four time periods depending on the year of birth of the child. Children born in 2010 (n= 279) were pooled with the 2009 (n=1, 452) cohort due to low sample size.

Aim 1 was to examine trends in adherence to the upper age limit restriction (of 105 and 223 days) for administration of rotavirus vaccination in children under 5 years of age in Nicaragua. Logistic regression (univariate) models were used to estimate crude odds ratios (O.R) for receiving rotavirus vaccination, among children receiving pentavalent vaccine after the age limit of (105 days and 223 days) for dose 1 and dose 3, respectively. These ORs were estimated separately for each cohort from 2006 to 2009. The outcome for these models was receipt of a rotavirus vaccination for dose 1 and dose 3.

Aim 2 was to identify factors that were associated with the receipt of rotavirus vaccine after the age limit. Logistic regression (multivariate) models were further adjusted for additional covariates to estimate an odds ratio (O.R) of receiving rotavirus vaccination, among children receiving pentavalent vaccine after the age limit of (105

days and 223 days) for dose 1 and dose 3, respectively, for the entire cohort. Potential confounders and other covariates were controlled for, where appropriate, based on a conceptual framework. Associations were presented as odds ratios (O.R) or adjusted O.R with their 95% Confidence Intervals. Statistical significance was set at an a priori p-value of <0.05. SAS version 9.4 (SAS Institute, Cary, N.C, USA) was used for statistical analysis.

3.5 Study Variables

Key variables for this research were the receipt and date of vaccination (rotavirus and pentavalent) as confirmed by medical record or by vaccine cards. Receipt of rotavirus vaccine was the outcome variable and age at time of receipt of pentavalent vaccine was the response variable. Covariates were obtained from a maternal interview at the time of enrolment. Child related variables consisted of date of birth, gender, and medical history (history of premature birth, duration of breastfeeding, chronic conditions, birth weight and history of HIV. Socioeconomic variables included education level of the mother, household size, mode of transportation and household possession of radio, television, computer and refrigerator. Demographic variables included maternal marital status and area of residence.

4. RESULTS

Table 1 describes the characteristics of the enrolled children and their households. Children from the entire cohort had a mean birth weight of 3.4 (1.3) kg with median (25^{th} and 75^{th} percentiles) reported duration of breast feed was 4 (3, 6) weeks. Eight percent of the children had a history of prematurity. The majority of the households (55%) belonged to the region of Managua. None of the variables were found to have statistically significant differences among the cohorts for each year.

Of 10,660 children, 1,887 met exclusion criteria. Thus, 8,773 children had received their 1^{st} dose of pentavalent and were age eligible to receive the 1^{st} and 3^{rd} dose of rotavirus. **Figure 1** is a flow diagram describing the entire cohort, in which 8,527 (97.2 %) children received their first dose of rotavirus and 7,385 (84.2 %) received their 3^{rd} dose of rotavirus vaccine. Median (25^{th} and 75^{th} percentiles) age of administration of dose 1 pentavalent and rotavirus vaccine receipt was 63 (61-69) days, and for dose 3 of pentavalent and rotavirus vaccine was 189 (184-209) days. A very high proportion of children (99 %) received their first dose of rotavirus and pentavalent on the same day and 99.6 % received their 3^{rd} dose of both vaccines on the same day. Among 246 children who did not receive their first rotavirus dose, 68 (27.7 %) received pentavalent vaccine after the age limit of 105 days. Among 1,388 children who did not receive their third dose of rotavirus vaccine, 280 (20.2 %) received their third dose of pentavalent vaccine after the age limit of 223 days of age.

Table 2 & Figure 2 show the number of children receiving rotavirus and pentavalent vaccine (dose 1) based on timing (before or after the age limit of 105 days). Delayed (beyond 105 days of age) receipt of pentavalent vaccine (dose 1) was strongly associated with non-receipt of rotavirus vaccine. In 2006, there were no children who received their first dose of rotavirus vaccine after the age limit of 105 days. In 2007, there were 127 children who received pentavalent vaccine after the age limit of 105 days, among them, 83 (65.4 %) received rotavirus vaccine. The proportion of children receiving rotavirus vaccine (dose 1) after the age limit increased in 2008 with (88 %) and 2009 with (92 %). **Table 3 & Figure 3** demonstrate the number of children receiving rotavirus and pentavalent vaccine (dose 3) based on timing (before or after the age limit of 223 days). In 2006, there were 40 children who received pentavalent vaccine after the age limit, among whom 27 (67.5 %) received rotavirus vaccine. Similar to data for dose 1, the proportion of children receiving rotavirus vaccine (dose 3) after 223 days remained consistent in 2007 (64 %), increased in 2008 (86.3 %) and 2009 with (94.7 %).

Among children receiving their 1st dose of pentavalent vaccine after the age limit of 105 days, the likelihood of simultaneously receiving rotavirus vaccine was decreased by 90 % (O.R = 0.1 (95%CI: 0.08, 0.16)) compared to children receiving their rotavirus vaccine before the age limit, as shown in **Table 4**. This pattern for dose 1 remained consistent over time. Among children receiving their 3rd dose of pentavalent vaccine after the age limit of 223 days, the likelihood of simultaneously receiving rotavirus vaccine was decreased by 40% (O.R = 0.6 (95%CI: 0.2, 0.8)) in 2006, 30 % (O.R = 0.7 (95% CI: 0.3, 0.4)) in 2007, 10 % (O.R = 0.9 (95% CI: 0.7, 1.2) in 2008 and (O.R = 3.8 (95%CI:

1.9, 7.2)) in 2009 compared to children receiving pentavalent vaccine before the age limit as shown in **Table 4.**

Findings from multivariate models examining additional predictors of rotavirus vaccination are shown in table 5. Children residing in Managua had a lower likelihood of receiving rotavirus vaccine after the age limit for the respective doses compared to children receiving their pentavalent vaccine before the age limits. For dose 1, the odds of receiving rotavirus vaccine after the age limit was 2.1 (95%CI: 1.2, 3.6) in Matagalpa, 10.7 (95%CI: 4.6, 24.6) in Masaya and 1.8 (95%CI: 1.1, 2.8) in Carazo compared to Managua (**Table 5**). For dose 3, the odds of receiving rotavirus vaccine after the age limit was higher in other regions such that children in Matagalpa region had 1.9 (95%CI: 1.6, 2.4) fold higher odds of receipt. The OR was 3.8 in Masaya (95%CI: 3.1, 4.7) and 2.8 (95%CI: 2.2, 3.5) in Carazo compared to Managua. Children with mothers who reached higher maternal education levels (adjusted O.R = 1.7 (1.3, 2.2)) and children who were not diagnosed with pneumonia (O.R = 1.5 (1.1, 2.2)) had a higher likelihood of receiving rotavirus vaccine after the age limit (**Table 6).**

5. DISCUSSION

These analyses suggest that the age restriction did lead to suboptimal vaccine coverage for rotavirus vaccine when compared with pentavalent coverage, though the coverage for the first dose of rotavirus vaccine improved with each new birth cohort. These findings are consistent with other studies (14, 45, 46, 48, 50). Persistent differences between pentavalent and rotavirus vaccine coverage after several years suggest that a substantial number of children did not receive rotavirus vaccine due to delayed presentation for vaccination rather than slow scale-up of the rotavirus immunization program. High coverage and timeliness of vaccinations indicate overall maturity of the program at the time of rotavirus vaccine introduction.

The adherence to the upper age restriction was strong throughout the study period for the first dose and was found to weaken over time for the third dose. One reason that might explain the strong adherence for dose 1 would be the prevailing concern of intussusception (peak onset of 3-4 months of age) (51). Weak adherence for dose 3 may have been due to the decreased risk of intussusception in older infants, as infants would have crossed the high risk period, coupled with increasing recognition of the need to prevent severe gastroenteritis and death due to rotavirus (42, 51).

Among children receiving their first and third dose of pentavalent vaccine after the age limit, children had a lower likelihood of receiving the corresponding rotavirus vaccine dose in Managua. Health care providers in Managua may have been better informed regarding recommendations, and trained on adhering to the restriction.

Health care providers in other cities appeared to be less adherent to the age restriction over time and the presence of transportation had no impact on receipt of rotavirus vaccine. These results are in contrast with findings by Flannery et al, (Brazil) that the difference between rotavirus and pentavalent vaccine coverage was greatest in regions that have lower routine immunization coverage, difficult-to-reach populations and historically higher diarrhea-related mortality (45). For the third dose, infants in this study whose mothers had a higher education level and children free of pneumonia had a higher likelihood of receiving rotavirus vaccine after the age limit. This correlates to findings in El Salvador by Suarez-Castaneda et al (46).

There were several strengths for this study. Assessment of associations was based on individual information of 8,773 children, rather than ecological or aggregated data. The analyses consisted of children from four regional sites in Nicaragua which may be generalizable to other low and middle income countries, especially in Latin America. Receipt of both vaccinations were confirmed using vaccination cards (96 %) and medical records (4 %) eliminating recall bias and misclassification errors that would have occurred if vaccine receipt was based on parental recall.

The study also had some limitations. All the covariates (birth weight, duration of breastfeeding, maternal education) were obtained from maternal interview, which is subject to reporting bias. No information was obtained about any rotavirus vaccine shortages reported in Nicaragua. However, rotavirus vaccine coverage was almost equal to pentavalent vaccine coverage when children presented to the clinic before the age limit as described in table 2 & 3. The large O.R 3.8 (95%CI: 1.9, 7.2) seen in the 2009

cohort (albeit with a wide confidence interval) could be related to a higher confidence with administration of rotavirus vaccine over time. Children in the 2009 cohort therefore had a 3.8 fold lower odds of not receiving their corresponding rotavirus vaccine (provided they received pentavalent before the age limit of 223 days) compared to those receiving it after the age limit. This could also suggest receipt of rotavirus vaccine became more independent of pentavalent vaccinations and less adherent to age restrictions, over time. This analysis did not include cohorts born before rotavirus vaccine introduction, so it was unable to assess improved timeliness in administration for other routine vaccines as observed in Australia (37).

These findings add to the limited literature about the use and coverage of rotavirus vaccine when there are age restrictions with its use. This was a unique time when such age limitations were recommended for vaccine administration. These analyses may potentially inform other country programs that are considering introducing vaccines associated with age restrictions. Findings also add to the growing number of investigations of vaccination timeliness and adherence to recommended ages for administration (37, 38). This message is consistent with the principle that all recommended vaccines should be administered during a vaccination visit to avoid missed opportunities and optimize vaccine coverage, especially for new vaccines.

6. REFERENCES

1. Bishop RF, Davidson GP, Holmes IH, Ruck BJ. Virus particles in epithelial cells of duodenal mucosa from children with acute non-bacterial gastroenteritis. Lancet 1973;2(7841):1281-3.
2. Flewett TH, Bryden AS, Davies H. Letter: Virus particles in gastroenteritis. Lancet 1973;2(7844):1497.
3. Tate JE, Burton AH, Boschi-Pinto C, Steele AD, Duque J, Parashar UD, et al. 2008 estimate of worldwide rotavirus-associated mortality in children younger than 5 years before the introduction of universal rotavirus vaccination programmes: a systematic review and meta-analysis. Lancet Infect Dis 2012;12(2):136-41.
4. Mwenda JM, Ntoto KM, Abebe A, Enweronu-Laryea C, Amina I, Mchomvu J, et al. Burden and epidemiology of rotavirus diarrhea in selected African countries: preliminary results from the African Rotavirus Surveillance Network. J Infect Dis 2010;202 Suppl:S5-S11.
5. Walker CLF, Rudan I, Liu L, Nair H, Theodoratou E, Bhutta ZA, et al. Global burden of childhood pneumonia and diarrhoea. Lancet 2013;381(9875):1405-16.
6. Kotloff KL, Nataro JP, Blackwelder WC, Nasrin D, Farag TH, Panchalingam S, et al. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. Lancet 2013;382(9888):209-22.
7. Wendy B. Vaccination with 3-dose paediatric rotavirus vaccine (RotaTeq(R)): impact on the timeliness of uptake of the primary course of DTPa vaccine. Vaccine 2012;30(35):5293-7.
8. de Palma O, Cruz L, Ramos H, de Baires A, Villatoro N, Pastor D, et al. Effectiveness of rotavirus vaccination against childhood diarrhoea in El Salvador: case-control study. BMJ 2010;340:c2825.
9. Cotes-Cantillo K, Paternina-Caicedo A, Coronell-Rodríguez W, Alvis-Guzmán N, Parashar UD, Patel M, et al. Effectiveness of the monovalent rotavirus vaccine in Colombia: a case-control study. Vaccine 2014;32(25):3035-40.
10. Cortese MM, Parashar UD, Control CfD, Prevention. Prevention of rotavirus gastroenteritis among infants and children: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 2009;58(RR-2):1-25.
11. Correia JB, Patel MM, Nakagomi O, Montenegro FMU, Germano EM, Correia NB, et al. Effectiveness of monovalent rotavirus vaccine (Rotarix) against severe diarrhea caused by serotypically unrelated G2P[4] strains in Brazil. J Infect Dis 2010;201(3):363-9.
12. Gray J, Iturriza-Gómara M. Rotaviruses. In: Stephenson JR, Warnes A, editors. Diagnostic Virology Protocols: Humana Press; 2011. p. 325-355.
13. Török TJ, Kilgore PE, Clarke MJ, Holman RC, Bresee JS, Glass RI. Visualizing geographic and temporal trends in rotavirus activity in the United States, 1991 to 1996. National Respiratory and Enteric Virus Surveillance System Collaborating Laboratories. Pediatr Infect Dis J 1997;16(10):941-6.
14. de Oliveira LH, Danovaro-Holliday MC, Sanwogou NJ, Ruiz-Matus C, Tambini G, Andrus JK. Progress in the introduction of the rotavirus vaccine in Latin America and the Caribbean: four years of accumulated experience. Pediatr Infect Dis J 2011;30(1 Suppl):S61-6.
15. Ward RL, Bernstein DI, Young EC, Sherwood JR, Knowlton DR, Schiff GM. Human rotavirus studies in volunteers: determination of infectious dose and serological response to infection. J Infect Dis 1986;154(5):871-80.
16. Kovacs A, Chan L, Hotrakitya C, Overturf G, Portnoy B. Rotavirus gastroenteritis. Clinical and laboratory features and use of the Rotazyme test. Am J Dis Child 1987;141(2):161-6.

17. Rodriguez WJ, Kim HW, Arrobio JO, Brandt CD, Chanock RM, Kapikian AZ, et al. Clinical features of acute gastroenteritis associated with human reovirus-like agent in infants and young children. J Pediatr 1977;91(2):188-93.
18. Kang G, Iturriza-Gomara M, Wheeler JG, Crystal P, Monica B, Ramani S, et al. Quantitation of group A rotavirus by real-time reverse-transcription-polymerase chain reaction: Correlation with clinical severity in children in South India. Journal of Medical Virology 2004;73(1):118-122.
19. King AE, Fleming DC, Critchley HOD, Kelly RW. Differential expression of the natural antimicrobials, beta-defensins 3 and 4, in human endometrium. Journal of Reproductive Immunology 2003;59(1):1-16.
20. Bhandari N, Rongsen-Chandola T, Bavdekar A, John J, Antony K, Taneja S, et al. Efficacy of a monovalent human-bovine (116E) rotavirus vaccine in Indian children in the second year of life. Vaccine 2014;32 Suppl 1:A110-6.
21. Control CfD, Prevention. Postmarketing monitoring of intussusception after RotaTeq vaccination--United States, February 1, 2006-February 15, 2007. MMWR Morb Mortal Wkly Rep 2007;56(10):218-22.
22. Groome MJ, Page N, Cortese MM, Moyes J, Zar HJ, Kapongo CN, et al. Effectiveness of monovalent human rotavirus vaccine against admission to hospital for acute rotavirus diarrhoea in South African children: a case-control study. Lancet Infect Dis 2014;14(11):1096-104.
23. Haber P, Patel M, Izurieta HS, Baggs J, Gargiullo P, Weintraub E, et al. Postlicensure monitoring of intussusception after RotaTeq vaccination in the United States, February 1, 2006, to September 25, 2007. Pediatrics 2008;121(6):1206-12.
24. Linhares AC, Velázquez FR, Pérez-Schael I, Sáez-Llorens X, Abate H, Espinoza F, et al. Efficacy and safety of an oral live attenuated human rotavirus vaccine against rotavirus gastroenteritis during the first 2 years of life in Latin American infants: a randomised, double-blind, placebo-controlled phase III study. Lancet 2008;371(9619):1181-9.
25. Madhi SA, Cunliffe NA, Steele D, Witte D, Kirsten M, Louw C, et al. Effect of human rotavirus vaccine on severe diarrhea in African infants. N Engl J Med 2010;362(4):289-98.
26. Patel MM, Haber P, Baggs J, Zuber P, Bines JE, Parashar UD. Intussusception and rotavirus vaccination: a review of the available evidence. Expert Rev Vaccines 2009;8(11):1555-64.
27. Vesikari T, Karvonen A, Ferrante SA, Ciarlet M. Efficacy of the pentavalent rotavirus vaccine, RotaTeq®, in Finnish infants up to 3 years of age: the Finnish Extension Study. Eur J Pediatr 2010;169(11):1379-86.
28. Orenstein WA, Bernier RH, Dondero TJ, Hinman AR, Marks JS, Bart KJ, et al. Field evaluation of vaccine efficacy. Bulletin of the World Health Organization 1985;63(6):1055-1068.
29. Madhi SA, Kirsten M, Louw C, Bos P, Aspinall S, Bouckenooghe A, et al. Efficacy and immunogenicity of two or three dose rotavirus-vaccine regimen in South African children over two consecutive rotavirus-seasons: a randomized, double-blind, placebo-controlled trial. Vaccine 2012;30 Suppl 1:A44-51.
30. Cunliffe NA, Witte D, Ngwira BM, Todd S, Bostock NJ, Turner AM, et al. Efficacy of human rotavirus vaccine against severe gastroenteritis in Malawian children in the first two years of life: a randomized, double-blind, placebo controlled trial. Vaccine 2012;30 Suppl 1:A36-43.
31. Doll MK, Buckeridge DL, Morrison KT, Gagneur A, Tapiero B, Charest H, et al. Effectiveness of monovalent rotavirus vaccine in a high-income, predominant-use setting. Vaccine 2015;33(51):7307-14.

32. 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.
33. Perez-Vilar S, Diez-Domingo J, Lopez-Lacort M, Martinez-Ubeda S, Martinez-Beneito MA. Effectiveness of rotavirus vaccines, licensed but not funded, against rotavirus hospitalizations in the Valencia Region, Spain. BMC Infect Dis 2015;15:92.
34. Bar-Zeev N, Kapanda L, Tate JE, Jere KC, Iturriza-Gomara M, Nakagomi O, et al. Effectiveness of a monovalent rotavirus vaccine in infants in Malawi after programmatic roll-out: an observational and case-control study. Lancet Infect Dis 2015;15(4):422-8.
35. Marlow R, Ferreira M, Cordeiro E, Trotter C, Januario L, Finn A, et al. Case control study of rotavirus vaccine effectiveness in Portugal during 6 years of private market use. Pediatr Infect Dis J 2015;34(5):509-12.
36. Premkumar PS, Parashar UD, Gastanaduy PA, McCracken JP, de Oliveira LH, Payne DC, et al. Reduced rotavirus vaccine effectiveness among children born during the rotavirus season: a pooled analysis of 5 case-control studies from the Americas. Clin Infect Dis 2015;60(7):1075-8.
37. Desai R, Oliveira LH, Parashar UD, Lopman B, Tate JE, Patel MM. Reduction in morbidity and mortality from childhood diarrhoeal disease after species A rotavirus vaccine introduction in Latin America - a review. Mem Inst Oswaldo Cruz 2011;106(8):907-11.
38. Becker-Dreps S, Paniagua M, Dominik R, Cao H, Shah NK, Morgan DR, et al. Changes in childhood diarrhea incidence in nicaragua following 3 years of universal infant rotavirus immunization. Pediatr Infect Dis J 2011;30(3):243-7.
39. Atherly D, Dreibelbis R, Parashar UD, Levin C, Wecker J, Rheingans RD. Rotavirus vaccination: cost-effectiveness and impact on child mortality in developing countries. J Infect Dis 2009;200 Suppl 1:S28-38.
40. Control CfD, Prevention. Suspension of rotavirus vaccine after reports of intussusception--United States, 1999. MMWR Morb Mortal Wkly Rep 2004;53(34):786-9.
41. Rothman KJ, Young-Xu Y, Arellano F. Age dependence of the relation between reassortant rotavirus vaccine (RotaShield) and intussusception. J Infect Dis 2006;193(6):898; author reply 898-9.
42. Murphy TV, Gargiullo PM, Massoudi MS, Nelson DB, Jumaan AO, Okoro CA, et al. Intussusception among infants given an oral rotavirus vaccine. N Engl J Med 2001;344(8):564-72.
43. Meeting of the Strategic Advisory Group of Experts on immunization, April 2012 – conclusions and recommendations; August 10, 2007. 2007;pp. 285–96. (Weekly epidemiological record 82).
44. (PAHO) PAHO. Paving the way for immunization; Meeting of the Technical Advisory Group on Vaccine-Preventable Diseases (TAG);Washington DC, USA. .
45. Flannery B, Samad S, de Moraes JC, Tate JE, Danovaro-Holliday MC, de Oliveira LH, et al. Uptake of oral rotavirus vaccine and timeliness of routine immunization in Brazil's National Immunization Program. Vaccine 2013;31(11):1523-8.
46. Suarez-Castaneda E, Burnett E, Elas M, Baltrons R, Pezzoli L, Flannery B, et al. Catching-up with pentavalent vaccine: Exploring reasons behind lower rotavirus vaccine coverage in El Salvador(). Vaccine 2015;33(48):6865-6870.
47. Suarez-Castaneda E, Pezzoli L, Elas M, Baltrons R, Crespin-Elias EO, Pleitez OA, et al. Routine childhood vaccination programme coverage, El Salvador, 2011-In search of timeliness. Vaccine 2014;32(4):437-44.
48. Patel MM, Clark AD, Sanderson CFB, Tate J, Parashar UD. Removing the age restrictions for rotavirus vaccination: a benefit-risk modeling analysis. PLoS Med 2012;9(10):e1001330.

49. (WHO) WHO. Meeting of the Strategic Advisory Group of Experts on immunization, April 2012 - conclusions and recommendations. Weekly Epidemiological Record;no.21(87).
50. A S, F P, M KA. Impact of rotavirus vaccination on coverage and timing of pentavalent vaccination - experience from two Latin American countries. Hum Vaccin Immunother 2016:0.
51. Murphy TV, Gargiullo PM, Wharton M. More on Rotavirus Vaccination and Intussusception. New England Journal of Medicine 2002;346(3):211-212.

7. TABLES / FIGURES

Table 1: Descriptive characteristics for child, socioeconomic and demographic related variables for each cohort.

	2006 (n=423)	2007 (n=3421)	2008 (n=3198)	2009 (n=1731)
Child Characteristics	**Mean (S.D)**	**Mean (S.D)**	**Mean (S.D)**	**Mean (S.D)**
Birth weight (kg)	3.4 (1.4)	3.5 (1.5)	3.3 (1.3)	3.2 (0.9)
	Median (Q1-Q3)	**Median (Q1-Q3)**	**Median (Q1-Q3)**	**Median (Q1-Q3)**
Breastfed (weeks)	4 (3, 6)	4 (3, 6)	4 (3, 6)	4 (3, 5)
	N (%)	**N (%)**	**N (%)**	**N (%)**
Sex: Male	221 (52.3)	1911 (55.9)	1726 (53.9)	985 (57)
Premature birth: Yes	35 (8.3)	303 (8.9)	263 (8.2)	142 (8.2)
Mother's education: None	21 (5)	125 (3.6)	111 (3.5)	79 (4.6)
Primary school	125 (30)	1022 (29.9)	977 (30.6)	57 (32.2)
Secondary school	225 (53.2)	1887 (55.2)	1737 (54.3)	901 (52.1)
Tertiary school	52 (12.3)	378 (11.1)	370 (11.6)	193 (11.2)
Region: Managua	256 (62.7)	2075 (60.6)	1683 (52.6)	842 (48.6)
Matagalpa	51 (12.1)	318 (9.3)	387 (12.1)	283 (16.4)
Masaya	71 (16.8)	599 (17.5)	693 (21.7)	350 (20.2)
Carazo	36 (8.5)	429 (12.5)	435 (13.6)	256 (14.8)

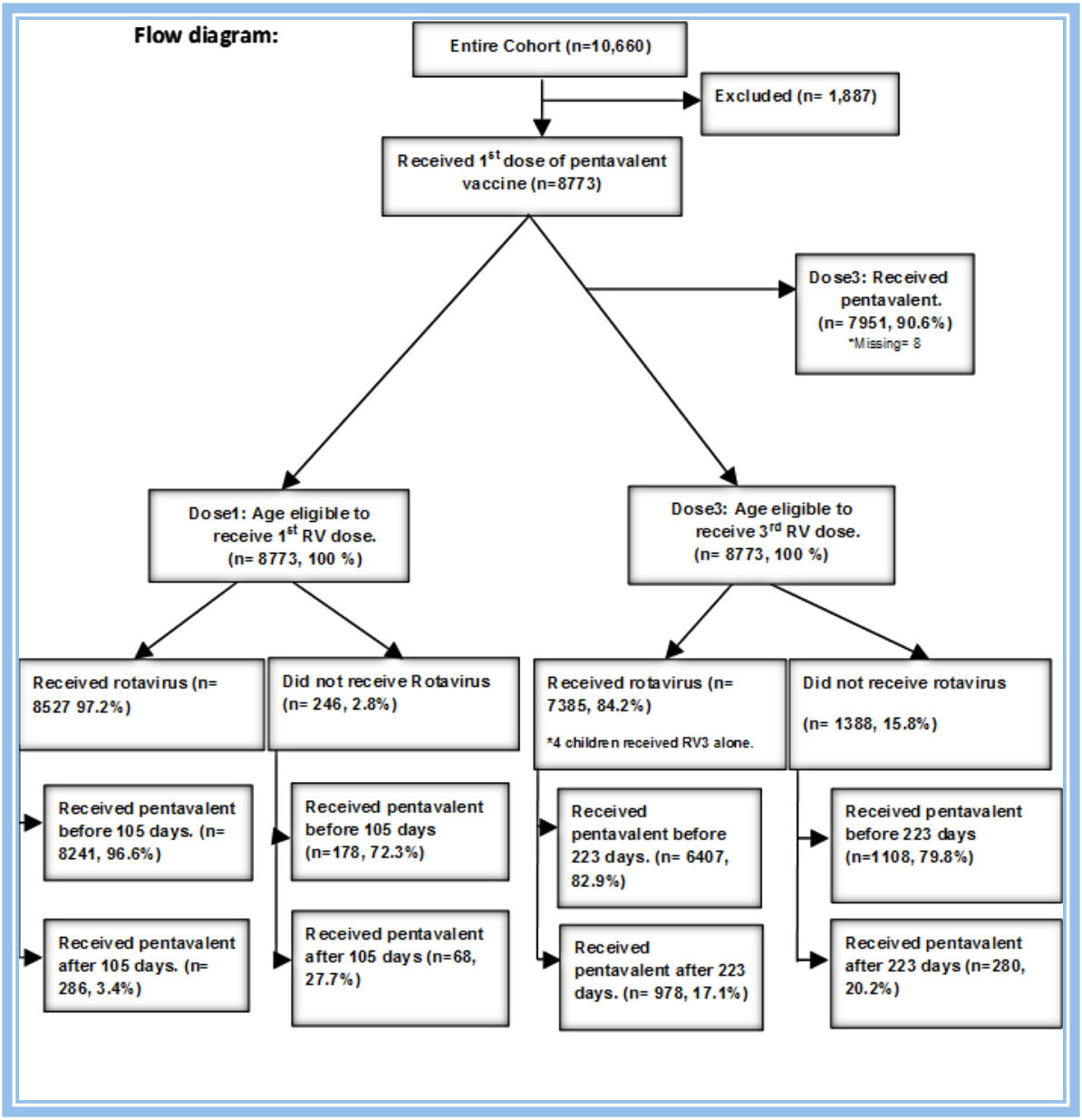

Figure 1: Flow chart describing the cohort of children used in this study. Among 10,660 children, 1,817 met the exclusion criteria leaving 8,773 in the analyses who had received their 1st dose of pentavalent vaccine and were age eligible to receive their 1st and 2nd dose of rotavirus vaccine.

Table 2: Proportion of children who received/not received Rotavirus vaccination (dose 1) before/after receiving pentavalent dose1 (before/after the age limit of 105 days).

Rotavirus (1st dose)	Received 1st dose of pentavalent before 105 days. Frequency (%)				Received 1st dose of pentavalent vaccine after 105 days. Frequency (%)			
	2006 (n=423)	**2007 (n=3294)**	**2008 (n=3057)**	**2009 (n=1645)**	**2006 (n=0)**	**2007 (n=127)**	**2008 (n=141)**	**2009 (n= 86)**
Not received	33 (7.8)	121 (3.7)	22 (0.7)	2 (0.1)	0	44 (33.6)	17 (12.1)	7 (8.1)
Received	390 (92.2)	3173 (96.3)	3035 (99.3)	1643 (99.9)	0	83 (65.4)	124 (87.9)	79 (91.9)

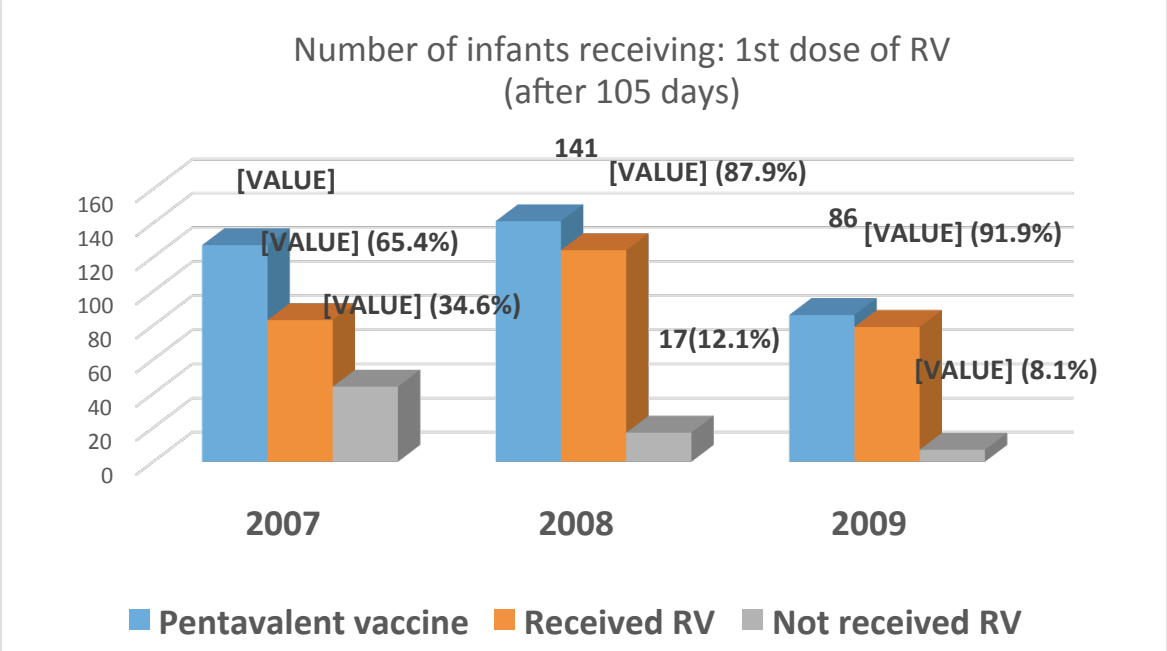

Figure 2: Proportion of children who received/not received rotavirus vaccination (dose 1) among those who received pentavalent (dose 1) vaccine (after the age limit of 105 days).

Table 3: Proportion of children who received/not received Rotavirus vaccination (dose 3) before/after pentavalent (dose 3) vaccine (before/after the age limit of 223 days).

Rotavirus (3rd dose)	Received 3rd dose of pentavalent before 223 days. Frequency (%)				Received 3rd dose of pentavalent vaccine after 223 days. Frequency (%)			
	2006 (n=383)	**2007 (n=2901)**	**2008 (n=2688)**	**2009 (n=1543)**	**2006 (n=40)**	**2007 (n=520)**	**2008 (n=510)**	**2009 (n= 188)**
Not received	58 (15.1)	447 (15.4)	333 (12.4)	270 (17.5)	13 (32.5)	187 (36)	70 (13.7)	10 (5.3)
Received	325 (84.9)	2454 (84.6)	2355 (87.6)	1273 (82.5)	27 (67.5)	333 (64)	440 (86.3)	178 (94.7)

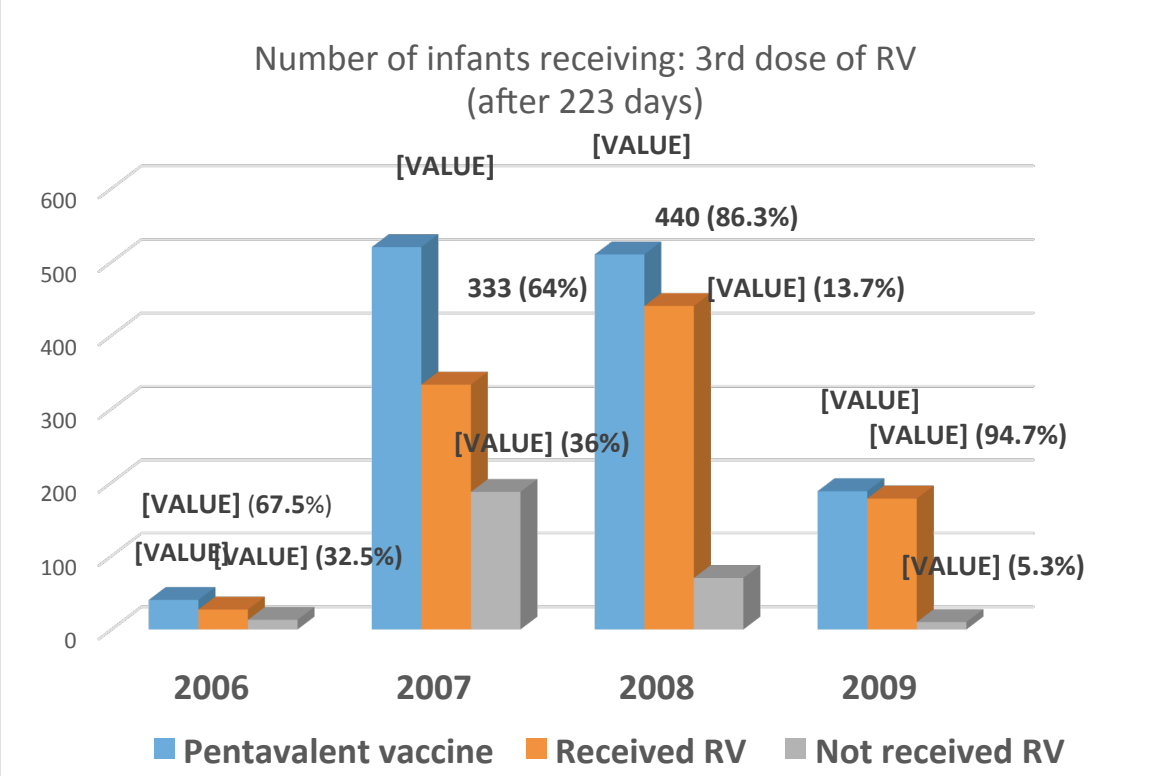

Figure 3: Proportion of children who received/not received Rotavirus vaccination (dose 3) among those who received pentavalent (dose 3) vaccine (after the age limit of 223 days).

Table 4: Univariate and multivariate regression results for the Odds (95% CI) of an infant receiving their first and third dose of rotavirus (who were vaccinated with pentavalent) after the age limit of 105 and 223 days for each cohort by year

Cohort	Odds ratio for rotavirus vaccination after the age limit of 105 days. Dose 1		Odds ratio for rotavirus vaccination after the age limit of 223 days. Dose 3	
	Crude 0.R (95 % CI)	Adjusted O.R (95 % CI)	Crude 0.R (95 % CI)	Adjusted O.R (95 % CI)
Pentavalent vaccination at the age limit (after vs before) ENTIRE COHORT)	0.10 (0.08, 0.16)	0.06 (0.05, 0.09)	0.6 (0.5, 0.7)	0.7 (0.6, 0.9)
2006	*	*	0.4 (0.2, 0.8)	0.5 (0.2, 1.0)
2007	0.07 (0.05, 0.10)	0.07 (0.05, 0.10)	0.3 (0.3, 0.4)	0.4 (0.3, 0.5)
2008	0.05 (0.02, 0.10)	0.06 (0.02, 0.10)	0.9 (0.7, 1.2)	1.2 (0.9, 1.6)
2009	0.01 (0.01, 0.07)	0.01 (0.01, 0.06)	3.8 (1.9, 7.2)	4.7 (2.4, 9.2)

*In 2006, there was no report of a child being administered with rotavirus vaccine after the age limit.

Table 5: Multivariate regression results to identify other risk factors, for the adjusted odds (95% CI) of an infant receiving their 1st dose of rotavirus vaccine (who were vaccinated with pentavalent after the age limit).

Predictors	Odds ratio for rotavirus vaccination (dose 1) after the age limit, 105 days)	
	Unit	Adjusted O.R (95 % CI)
Pentavalent vaccination at the age limit (after vs before)	Received pentavalent vaccine after 105 days vs before 105 days of age.	0.06 (0.05, 0.09)
Sex	Female vs male	1.3 (0.9, 1.7)
Region (Reference = Managua)	Matagalpa vs Managua	2.1 (1.2, 3.6)
	Masaya vs Managua	10.7 (4.6, 24.6)
	Carazo vs Managua	1.8 (1.1, 2.8)

Table 6: Multivariate regression results to identify other risk factors, for the odds (95% CI) of an infant receiving their 3rd dose of rotavirus vaccine (who were vaccinated with pentavalent vaccine after the age limit).

Predictors	**Odds ratio for rotavirus vaccination (dose 3) after the age limit, 223 days)**	
	Unit	Adjusted O.R (95 % CI)
Pentavalent vaccination at the age limit (after vs before)	Received pentavalent vaccine after 223 days of age vs before 223 days of age.	0.7 (0.6, 0.9)
Sex	Female vs male	1.1 (0.9, 1.3)
Region (Reference = Managua)	Matagalpa vs Managua	1.9 (1.6, 2.4)
	Masaya vs Managua	3.8 (3.1, 4.7)
	Carazo vs Managua	2.8 (2.2, 3.5)
Maternal education	None vs primary school	0.6 (0.5, 0.9)
	Secondary school vs primary school	1.2 (1.1, 1.4)
	Tertiary school vs primary school	1.7 (1.3, 2.2)
Pneumonia	No pneumonia vs pneumonia	1.5 (1.1, 2.2)
Car	Car vs No car	1.2 (0.9, 1.5)