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Efficacy of human rotavirus vaccine against severe gastroenteritis in Malawian children in the first two years of life: a randomised, double-blind, placebo controlled trial

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Abstract

Rotavirus gastroenteritis is a major cause of morbidity and mortality among African infants and young children. A phase III, placebo-controlled, multi-centre clinical trial of a live, oral G1P[8] human rotavirus vaccine (RIX4414) undertaken in Malawi and South Africa significantly reduced the incidence of severe rotavirus gastroenteritis in the first year of life. We now report on vaccine efficacy in the Malawi cohort of children who were followed into the second year of life. A total of 1,773 healthy infants were enrolled in Blantyre, Malawi into three groups. Two groups received three doses of RIX4414 or placebo at age 6, 10, and 14 weeks and the third group received placebo at 6 weeks and RIX4414 at age 10 and 14 weeks. Subjects were followed by weekly home visits for episodes of gastroenteritis until 1 year of age, and were then re-consented for further follow-up to 18-24 months of age. Severity of gastroenteritis episodes was graded according to the Vesikari scoring system. Seroconversion for anti-rotavirus IgA was determined on a subset of children by using ELISA on pre- and post-vaccine blood samples. Rotavirus VP7 (G) and VP4 (P) genotypes were determined by RT-PCR. A total of 70/1030 (6.8%, 95% CI 5.3 - 8.5) subjects in the pooled (2 dose plus 3 dose) RIX4414 group compared with 53/483 (11.0%, 8.3 - 14.1) subjects in the placebo group developed severe rotavirus gastroenteritis in the entire follow-up period (Vaccine Efficacy 38.1% (9.8 - 57.3)). The point estimate of efficacy in the second year of life (17.6%; -59.2 - 56.0) was lower than in the first year of life (49.4%; 19.2 - 68.3). There were non-significant trends towards a higher efficacy in the second year of life among children who

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Author Contributions: Nigel Cunliffe was the Principle Investigator of this study. The Malawi-based investigator team of Desiree Witte, Bagrey Ngwira, Stacy Todd, Nancy Bostock, Ann Turner, and Philips Chimpeni supervised enrolment and follow-up of subjects and collection of clinical data. Alain Bouckennooghe and A Duncan Steele conceived the idea of a clinical trial of *Rotarix* in Africa and provided expert advice through the trial, together with Kathleen Neuzil and John C Victor. Nigel Cunliffe drafted the manuscript with scientific input from all authors. All authors approved the final version of the manuscript.

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received the three-dose schedule compared with the two-dose schedule, and a higher anti-rotavirus IgA seroresponse rate in the three-dose RIX4414 group. Rotavirus strains detected included genotype G12 (31%); G9 (23%); and G8 (18%); only 18% of strains belonged to the G1P[8] genotype. While the optimal dosing schedule of RIX4414 in African infants requires further investigation, vaccination with RIX4414 significantly reduced the incidence of severe gastroenteritis caused by diverse rotavirus strains in an impoverished African population with high rotavirus disease burden in the first two years of life.

Keywords

Gastroenteritis; Rotavirus; Vaccine; Genotypes

Introduction

Diarrhoeal disease continues to represent a major threat to global child health, and was recently estimated to account for 15% of all deaths among children below 5 years of age [1]. Rotavirus is the most important aetiological agent of severe gastroenteritis, and is responsible for an estimated 527,000 childhood deaths annually [2], with over 250,000 rotavirus deaths occurring in the African continent [3, 4]. Hence, rotavirus disease prevention in Africa through vaccination is a public health priority [5].

Two live, oral, attenuated rotavirus vaccines are globally licensed for the prevention of rotavirus gastroenteritis. These include a monovalent serotype G1P[8] human rotavirus vaccine RIX4414 (*Rotarix*, GSK Biologicals, Belgium) and a multivalent, human-bovine reassortant rotavirus vaccine (*RotaTeq*, Merck & Co, USA) which contains the most common human rotavirus G-types (G1-G4), and P[8], the most common human rotavirus P-type. Both vaccines are highly efficacious in preventing severe rotavirus gastroenteritis in infancy in high and middle income countries [6-8], and in 2006 were recommended by WHO for use in the Americas and Europe where evidence of efficacy had been demonstrated [9]. In countries that have adopted rotavirus vaccine in their childhood immunization programmes, evidence of impact has been striking [10]. Importantly, evidence of reduction of diarrhoea deaths following routine rotavirus vaccination has recently been published from Mexico [11]. Finally, a recent study of *Rotarix* from Mexico and Brazil has documented that the benefit of routine rotavirus vaccination (reduction in childhood diarrhoea hospitalisations and deaths) far outweighs a small, short term risk of intussusception that may be associated with use of this live, oral vaccine [12].

In 2009, following review of vaccine performance in Africa and resource-poor settings in Latin America, a global recommendation for rotavirus vaccine use was issued [13]. This recommendation was in part informed by the results of a Phase III, placebo-controlled clinical trial of RIX4414 undertaken in Malawi and South Africa [14]. In this study, vaccination with RIX4414 significantly reduced severe rotavirus gastroenteritis episodes in the first year of life in both settings, although efficacy was lower in Malawi (49.4% [95% CI 19.2 – 68.3]) compared with South Africa (76.9% [56.0 – 88.4]). Notable findings in Malawi included a high incidence of severe rotavirus disease, a wide diversity of circulating rotavirus strains and a high exposure to natural rotavirus infection early in infancy [14]. This manuscript reports on vaccine performance and circulating rotavirus strains in Malawian children for an extended period of up to 24 months of age.

Methods

Study Design

A phase III, double-blind, randomized, placebo-controlled multicenter study was undertaken in South Africa and Malawi as previously reported [14]. In Malawi, children were enrolled in four health centres in Blantyre, the largest city in the Southern region of the country. Healthy infants were randomized at their first Expanded Program on Immunization (EPI) clinic visit into three groups. One group received three doses of placebo at 6, 10, and 14 weeks of age and a second group received three doses of RIX4414 at the same age. The third group received placebo at 6 weeks and RIX4414 at 10 and 14 weeks. The study was designed to reflect, as far as possible, the conditions under which rotavirus vaccine would be administered under “real-life” conditions in a typical African infant population. Thus, all EPI vaccines including oral poliovirus vaccine (OPV) were co-administered; HIV-infected or -exposed infants were included; and no restriction on breastfeeding around the time of vaccination was imposed.

Enrolment and Follow-up

Enrolment was conducted between October 2006 and July 2007. Subjects were initially followed-up until 12 months of age [14]. At age 1 year, parents/guardians were given the opportunity to enter their children into a period of extended follow-up, the conclusion of which was subject to a time cut-off of January 2009. Subjects were seen at the study clinic at the time of vaccination (~ 6, 10 and 14 weeks of age), at one month following the third dose of vaccine/placebo (~ age 18 weeks of age), at one year of age and, for those subjects who agreed to follow-up beyond one year, at final visit (18-24 months of age). In addition, study staff visited the subjects' homes at weekly intervals throughout the study period. Parents were encouraged to bring the subjects to clinic in the event of illness (unscheduled visits). In the case of severe illness requiring inpatient care, children were hospitalized at the Queen Elizabeth Central Hospital (QECH), a tertiary referral hospital in Blantyre. Voluntary testing of infants for HIV infection using ELISA and PCR was undertaken as previously described [14].

Assessment and Investigation of Gastroenteritis Episodes

Gastroenteritis was defined as the passage of three or more looser-than-normal stools in a 24 hour period, with or without vomiting. Parents completed a diary card for each gastroenteritis episode, the severity of which was graded according to the Vesikari scoring system with severe disease defined by a score of ≥ 11 [15]. Parents were asked to collect a stool specimen as soon as possible after the onset of gastroenteritis. Stool samples were frozen at -70°C until shipped to GSK Biologicals, Rixensart, Belgium for rotavirus testing by ELISA (*Rotaclo*ne, Meridian Biosciences, Cincinnati, OH), following which G and P types were determined at DDL Diagnostic Laboratory (Voorburg, the Netherlands) by a testing algorithm using RT-PCR and reverse hybridization [16].

Vaccine immunogenicity

Serum for anti-rotavirus IgA determination was obtained immediately prior to administration of the first dose of vaccine/placebo in a ~ 10% systematically selected subset of subjects (at ~ 6 weeks of age) and at one month following receipt of the third vaccine/placebo dose in all subjects (at ~ 18 weeks of age). Serum was frozen at -20°C prior to investigation for anti-rotavirus IgA by ELISA (GSK Biologicals), with an assay cut-off at 20U/ml. Seroconversion was defined as the presence of a demonstrable IgA titre at one month post-vaccination, in those infants without demonstrable pre-vaccination antibody.

Statistical Analysis

Infants who had received the complete vaccination course and had entered the efficacy surveillance period comprised the According-to-Protocol (ATP) efficacy cohort. Efficacy analysis began at 2 weeks after receipt of the 3rd dose of vaccine/placebo, and finished at final follow-up visit (age 18-24 months). The primary endpoint was the assessment of pooled vaccine efficacy (two dose RIX4414 plus three dose RIX4414) against severe rotavirus gastroenteritis up to one year of age for the combined Malawi and South African populations [14]. Supplementary analyses were performed to calculate country-specific vaccine efficacy against severe- and any severity- rotavirus gastroenteritis and severe all-cause gastroenteritis, in children followed up to 2 years of age, for the two- and three-dose schedules. All subjects who agreed to follow up beyond one year of age and who complied with the study protocol were included in the supplementary analyses, regardless of event(s) in the first year of life. Vaccine efficacy against a particular event was calculated using the formula $VE = (1 - \text{relative risk}) \times 100$, where relative risk = cumulative incidence of the event in the vaccinated group/cumulative incidence of the event in the placebo group. Ninety-five percent confidence intervals for vaccine efficacy were derived from the exact confidence interval for the Poisson rate ratio for each analysis [17]. A p-value was also calculated using a two-sided Fisher's exact test. The incidence rate in a group was computed as the number of infants reporting at least one event (the first event only was included) divided by the total follow-up time for each parameter or subgroup with corresponding 95% confidence intervals [18]. The number of events prevented (per 100 infants per year) was obtained as 100 times the difference in incidence rate between the group that received placebo and the group that received RIX4414. The associated confidence interval was derived using the method conceptualized by Zou and Donner [19].

Ethics

The study was undertaken according to Good Clinical Practice (GCP) guidelines. Informed consent was obtained from the subject's parent/guardian prior to any study procedure being undertaken. In case of illiteracy of the parent/guardian, consent was undertaken with the assistance of an impartial witness.

The study protocol was approved by the Malawi National Health Sciences Research Committee, the Liverpool School of Tropical Medicine Research Ethics Committee, and the ethics committee of the World Health Organisation.

Results

Study population

A total of 1,773 infants were enrolled in Malawi. Of these, 1,513 and 1,194 infants were included in the ATP efficacy cohorts for the first and second years of follow-up respectively (Figure 1). Demographic details were similar for vaccine and placebo groups [14]. The mean age (SD) at final visit was 19 months (4.78) for the RIX4414 group and 18.9 months (5.03) for the placebo group. The mean duration of follow-up was 0.6 years for the first follow-up period, 0.78 years for the second follow-up period and 1.25 years for the entire follow-up period.

Vaccine efficacy vs. rotavirus gastroenteritis

The incidence of severe rotavirus gastroenteritis was higher in the placebo group during the first year of follow-up (7.9%, 95% CI 5.6 – 10.6) than in the second year of follow-up (4.5%, 2.6 – 7.1) [Table 1]. Fewer episodes of severe rotavirus gastroenteritis occurred in the pooled RIX4414 group compared with the placebo group for the first, second, and entire follow-up periods (VE 49.4% [19.2 – 68.3], 17.6% [–59.2 – 56.0] and 38.1% [9.8 – 57.3]

respectively), although the differences were not statistically significant for the second follow-up period. For two years of follow-up, rotavirus vaccination prevented 6.5 episodes of severe rotavirus gastroenteritis per 100 infant years (Table 2). There was a trend towards greater protection against severe rotavirus gastroenteritis in the three-dose RIX4414 group compared with the two-dose RIX4414 group beyond the first year of life, although the study was not powered to detect differences between these two groups (Tables 1 and 2).

Vaccine efficacy vs. all-cause gastroenteritis

Vaccine efficacy against severe gastroenteritis of any cause was 25.1% (4.7 – 40.8) in the first year, 9.3% (–22.6 – 32.3) in the second year and 15.9% (–2.7 – 30.9) for the combined follow-up period (Table 3).

Immunogenicity

Among infants who had a pre-vaccination blood draw, 17 of 126 (13.5%) in the pooled vaccine group and 7 of 67 (10.4%) in the placebo group met the definition for seropositive, based on anti-rotavirus IgA antibody concentrations ≥ 20 u/ml. A total of 40.5% (25% - 57%) subjects in the placebo group ($n = 42$) and 52.9% (42% - 64%) of subjects in the pooled RIX4414 group ($n = 85$) seroconverted for anti-rotavirus IgA by approximately 18 weeks of age, with a non-significant higher rate of seroconversion in the 3-dose RIX4414 group (57.1%; 42% - 72%) compared with the 2-dose RIX4414 group [47.2%, 30% - 64%] (Figure 2). Post-vaccine/placebo GMC anti-rotavirus IgA titres (U/ml) were 38.2 (21- 68) in the placebo group compared with 57.8 (38-88), 63.0 (36-109) and 51.5 (26-102) in the pooled RIX4414, 3-dose RIX4414 and 2-dose RIX4414 groups respectively (Figure 2).

Rotavirus strains

Non-vaccine containing rotavirus serotypes predominated during the study period (Figure 3). Serotype G12 was the most prevalent strain type and comprised 31% of all strains, followed by serotypes G9 (23%) and G8 (18%). The G1P[8] strain comprised 18% of all strains.

Discussion

In this placebo-controlled clinical trial, the human rotavirus vaccine (RIX4414) significantly reduced the incidence of severe rotavirus gastroenteritis in Malawian children in the first two years of life. The relatively modest degree of protection observed (vaccine efficacy, 38.1%), should be interpreted in the context of an impoverished population with a high incidence of severe rotavirus gastroenteritis, a wide diversity of circulating rotavirus strains, concomitant administration of OPV, no restriction of breastfeeding at the time of vaccination, and the inclusion of HIV-exposed infants. Although the data are not directly comparable because of differences in study design, the efficacy point estimate in Malawi is similar to the reported efficacy in the first two years of life (39.3%) of the pentavalent rotavirus vaccine *RotaTeq* in a clinical trial recently undertaken in Ghana, Kenya and Mali [20], and to the efficacy of *RotaTeq* (42.7%) in a recent study undertaken in Bangladesh [21]. The high incidence of severe rotavirus gastroenteritis among children in both years of follow-up in Malawi, when considered together with sentinel hospital surveillance at the QECH, Blantyre confirming that rotavirus is responsible for a third of admissions for diarrhoea [22], suggests that a substantial public health impact of routine rotavirus vaccination in Malawi could be expected. An impact on severe gastroenteritis of any cause was also documented in this study. These data therefore support the WHO recommendation that rotavirus vaccine should be included in childhood immunisation programmes in this region [13].

Vaccine efficacy in Malawi was lower in the second year of life (17.6%) compared with the first year of life (49.4%), although the study was not designed to measure statistically significant efficacy during the second year of life. Nevertheless, a similar observation was reported from the South Africa site of this trial, with vaccine efficacies of 77% and 40% during the first and second years of the study, respectively [23], and in the *RotaTeq* trial in Africa, where vaccine efficacy was reported as 64.2% in the first year of life and 19.6% in the second year [20]. A lower vaccine efficacy after 12 months of age has also been suggested in post-introduction effectiveness studies of *Rotarix* in resource-poor settings in Brazil [24] and El Salvador [25], and has also been noted in Australian children [26]. It has been hypothesised that this phenomenon could be explained by waning immunity, and that it may be particularly pronounced when rotavirus strains heterotypic to the vaccine strain are circulating [24-26]. The hypothesis that waning immunity may be a factor in an apparent lower vaccine efficacy after 12 months of age in the current study is supported by the observation of a trend towards higher efficacy against severe rotavirus gastroenteritis in the second year of life provided by the three-dose RIX4414 schedule, combined with slightly higher antirotavirus IgA seroconversion rates and GMC titres in the three-dose compared with the two-dose RIX4414 group. However, it should be cautioned that this study was not powered to examine differences between the two- and three-dose vaccine schedules, and that the confidence intervals around the point efficacy estimate corresponding to each of these two schedules overlap. The potential benefit of a third vaccine dose therefore requires further investigation.

Since exposure to natural rotavirus infection confers protection against the subsequent development of severe rotavirus disease [27], a reduced efficacy in the second year of life in this study could also be partly explained by exposure of the placebo group to natural rotavirus infection in the first year of life. Because rotavirus circulates year-round in Malawi [22] the timing of enrolment was not determined by rotavirus season. Thus, 40.4% of the placebo group had serological evidence of exposure to natural rotavirus infection by one month post vaccination (~ 18 weeks of age) [14]. Of note, however, a recent study from Vellore, India, reported that protection against rotavirus diarrhoea occurring as a result of natural rotavirus infection was lower than documented in other settings, which may in part explain the reduced rotavirus vaccine performance observed in similar settings in Asia and Africa [28]. The high burden of severe rotavirus disease in the second year of life documented in the current study emphasises the importance of continued protection, and the potential public health value that even a modestly efficacious vaccine would bring if incorporated into Malawi's national immunisation programme.

Vaccine efficacy in Malawi was substantially lower than observed in clinical trials of both *Rotarix* and *RotaTeq* in upper & middle income countries, where an efficacy of 85-100% against severe rotavirus gastroenteritis had been demonstrated in the first year of life [6-8], and where protection is relatively well conserved into the second year of life [29-32]. Potential reasons (e.g. maternal antibodies, breastfeeding, concurrent OPV administration, malnutrition, concomitant HIV infection, rotavirus strain diversity, enteric co-infections, "force of infection") why the efficacy of live, oral rotavirus vaccines may be lower in developing countries have been discussed previously, but remain incompletely understood [33-35]. In our study, all mothers were breastfeeding, and > 99% of infants received concomitant OPV [14]. Less than 5% of enrolled infants were HIV infected [14]. The impact of nutritional status on vaccine efficacy and the role of concurrent infection with other enteric pathogens in this study cohort is currently being explored. Although there is no reliable, consistent laboratory correlate that predicts clinical protection following rotavirus vaccination [36, 37], it is known that the serum immune response to rotavirus vaccines decreases by income level of country [33]. The anti-rotavirus IgA seroconversion rate following vaccination in this study, 52.9%, is one of the lowest reported for *Rotarix* [33]. In

this regard, it is worthy of mention that Malawi is a very low income country (Gross National Income per capita of \$810 per annum) with an under 5 mortality rate of 100 per 1,000 live births (<http://www.who.int/gho/countries/mwi.pdf>).

A particular feature of this study was the diversity of circulating strains encountered, including genotypes G8, G9 and G12, with only a minority of strains carrying the G1P[8] genotype on which the vaccine is based. Surveillance of rotavirus strains undertaken in Malawi since 1997 has described an extraordinary diversity of rotaviruses [22], and African countries are known to harbour a wide variety of rotavirus strains [38]. The diversity of circulating strains documented during this study, when examined further at the whole genomic level, does not however explain the reduced vaccine efficacy in Malawi [39]. Furthermore, vaccine efficacy was consistent across strain types in both Malawian and South African populations [14, 40]. When considered together with the protection observed against a broad range of strain types by the pentavalent vaccine *RotaTeq* during recent studies in Africa and Asia [20, 21], these data suggest that rotavirus diversity *per se* is not a critical determinant of vaccine efficacy.

Although this study was undertaken to reflect some of the conditions of routine vaccine use, it will be important to examine vaccine performance when used in the childhood immunisation programme in Malawi. Vaccine effectiveness using a two-dose schedule of *Rotarix* administered at 6 & 10 weeks of age (the schedule recommended by WHO but not previously evaluated in a clinical trial) is being investigated in an effectiveness trial in Bangladesh (www.clinicaltrials.gov). The relationship between vaccine performance and age of administration also needs further assessment, in order to better understand the duration of protection provided by a two-dose schedule. Furthermore, although the vaccine efficacy (individual protection) in this clinical trial was relatively modest, the potential for an additive, indirect population benefit of vaccination is highlighted by recent experience from industrialised countries where greater than anticipated reductions in disease burden have been documented [41].

The protection provided by RIX4414 against severe rotavirus gastroenteritis in an impoverished African population is a major advance in the effort to reduce the global burden of rotavirus disease, over 20 years since clinical trials of early generation rotavirus vaccines undertaken in Africa failed to demonstrate an impact on rotavirus gastroenteritis (reviewed in [35]). Preliminary health economic analyses support the introduction of rotavirus vaccines in Malawi [42]. Introduction of this life-saving vaccine into Malawi and other countries with high rotavirus disease burden is urgently needed.

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Figure 1. Study assignment and follow-up of subjects for first and second follow-up periods.

Figure 2. Anti-rotavirus IgA antibody seroconversion rates and GMCs (95% Confidence Intervals) one month after third dose of Rotarix.

Seroconversion rate calculated on subset (~10%) of subjects who had pre-vaccination blood draws and who were seronegative for rotavirus antibody prior to dose 1 of vaccine.

Figure 3. Distribution of major strains causing severe rotavirus gastroenteritis episodes in placebo group from two weeks post-dose 3 until two years of age.

Table 1
Proportion of infants reporting at least one severe rotavirus gastroenteritis episode and vaccine efficacy against severe rotavirus gastroenteritis (according-to-protocol cohort for efficacy)

	Severe rotavirus gastroenteritis (95% CI)	Vaccine efficacy (95% CI)	p value
First year [*]			
Pooled	41/1030 (4.0%; 2.9–5.4)	49.4% (19.2–68.3)	0.003
Two-dose	21/525 (4.0%; 2.5 – 6.0)	49.2% (11.1 –71.7)	0.01
Three-dose	20/505 (4.0%; 2.4 –6.1)	49.7% (11.3 –72.2)	0.01
Placebo	38/483 (7.9%; 5.6–10.6)	-	-
Second year [†]			
Pooled	30/814 (3.7%; 2.5–5.2)	17.6% (–59.2–56.0)	0.619
Two-dose	18/413 (4.4%; 2.6 – 6.8)	2.6% (–101.2 –52.6)	1.000
Three-dose	12/401 (3.0%; 1.6 –5.2)	33.1% (–48.6 –70.9)	0.374
Placebo	17/380 (4.5%; 2.6–7.1)	-	-
Entire follow-up period [‡]			
Pooled	70/1030 (6.8%; 5.3–8.5)	38.1% (9.8–57.3)	0.012
Two-dose	38/525 (7.2%; 5.2–9.8)	34.0% (–2 –57.7)	0.062
Three-dose	32/505 (6.3%, 4.4 –8.8)	42.3% (8.8 –64.0)	0.017
Placebo	53/483 (11.0%; 8.3–14.1)	-	-

Data are n/N (%), unless otherwise indicated, where n = number of subjects reporting at least one event in each group and N = number of subjects included in each group.

^{*} Mean duration 0.6 years.

[†] Mean duration 0.78 years.

[‡] Mean duration 1.26 years for RIX4414 and 1.25 years for placebo.

Table 2
Risk of gastroenteritis in the RIX4414 group and the placebo group (according-to-protocol cohort for efficacy)

RIX4414			Placebo		Difference in rate (95% CI) [†]
	No. in cohort	Episodes per 100 Infants-Yr (95% CI)	No. in cohort	Episodes per 100 Infants-Yr (95% CI)	
Severe rotavirus gastroenteritis					
First Year					
Pooled	1030	6.5 (4.8–8.8)	483	13.1 (9.6–18.0)	6.7 (2.4–11.9)
Two-dose	525	6.5 (4.2–10.0)	-	-	6.6 (1.7–12.0)
Three-dose	505	6.4 (4.2–10.0)	-	-	6.7 (2.4–11.9)
Second Year					
Pooled	814	4.9 (3.4–7.0)	380	5.9 (3.7–9.5)	1.0 (–2.1–4.9)
Two-dose	413	5.8 (3.6–9.2)	-	-	0.1 (–3.9–4.3)
Three-dose	401	4.0 (2.3–7.0)	-	-	1.9 (–1.8–5.9)
Entire follow-up period					
Pooled	1030	9.8 (7.7–12.4)	483	16.3 (12.4–21.3)	6.5 (1.9–11.9)
Two-dose	525	10.4 (7.6–14.3)	-	-	5.9 (0.4–11.7)
Three-dose	505	9.2 (6.5–12.9)	-	-	7.1 (1.7–12.8)
Any rotavirus gastroenteritis					
First Year					
Pooled	1030	13.7 (11.1–17.0)	483	21.7 (16.9–27.9)	7.9 (2.1–14.6)
Two-dose	525	14.3 (10.6–19.1)	-	-	7.5 (0.6–14.6)
Three-dose	505	13.2 (9.7–18.0)	-	-	8.5 (1.7–15.6)
Second Year					
Pooled	814	8.3 (6.3–10.9)	380	6.9 (4.5–10.8)	–1.3 (–4.9–3.0)
Two-dose	413	8.6 (5.9–12.6)	-	-	–1.7 (–6.4–3.0)
Three-dose	401	7.9 (5.3–11.8)	-	-	–0.9 (–5.6–3.6)
Entire follow-up period					
Pooled	1030	18.7 (15.8–22.2)	483	25.0 (20.1–31.2)	6.3 (0.2–13.1)
Two-dose	525	19.3 (15.3–24.5)	-	-	5.7 (–1.4–13.1)
Three-dose	505	18.1 (14.1–23.2)	-	-	6.9 (–0.2–14.3)

[†] The difference in rate is calculated as the number of episodes per 100 infants per year in the placebo group minus the number of episodes per 100 infants per year in the RIX4414 group.

Table 3
Proportion of infants reporting at least one severe all-cause gastroenteritis episode and vaccine efficacy against severe all-cause gastroenteritis (according-to-protocol cohort for efficacy)

	Severe all-cause gastroenteritis (95% CI)	Vaccine efficacy (95% CI)	p value
First year [*]			
Pooled	187/1030 (18.2%; 15.8-20.6)	25.1% (4.7 – 40.8)	0.007
Two-dose	98/525 (18.7%;15.4-22.3)	22.9% (–1.7-41.7)	0.038
Three-dose	89/505 (17.6%;14.4-21.2)	27.2% (3.3-45.4)	0.012
Placebo	117/483 (24.2%; 20.5 – 28.3)	-	-
Second year [†]			
Pooled	138/814 (17.0%;14.4-19.7)	9.3% (–22.6 – 32.3)	0.304
Two-dose	67/413 (16.2%;12.8-20.1)	13.2% (–23.0-38.8)	0.456
Three-dose	71/401 (17.7%;14.1-21.8)	5.2% (–33.6-32.8)	0.812
Placebo	71/380 (18.7%;14.9-23.0)	-	-
Entire follow-up period [‡]			
Pooled	287/1030 (27.9%;25.1- 30.7)	15.9% (–2.7 – 30.9)	0.090
Two-dose	146/525 (27.8%; 24.0-31.9)	16.0% (–5.7 – 33.4)	0.141
Three-dose	141/505 (27.9%;24.0-32.1)	15.7% (–6.4 – 33.3)	0.154
Placebo	160/483 (33.1%;28.9-37.5)	-	-

Data are n/N (%), unless otherwise indicated, where n = number of subjects reporting at least one event in each group and N = number of subjects included in each group.

^{*} Mean duration 0.6 years.

[†] Mean duration 0.78 years.

[‡] Mean duration 1.26 years for RIX4414 and 1.25 years for placebo.