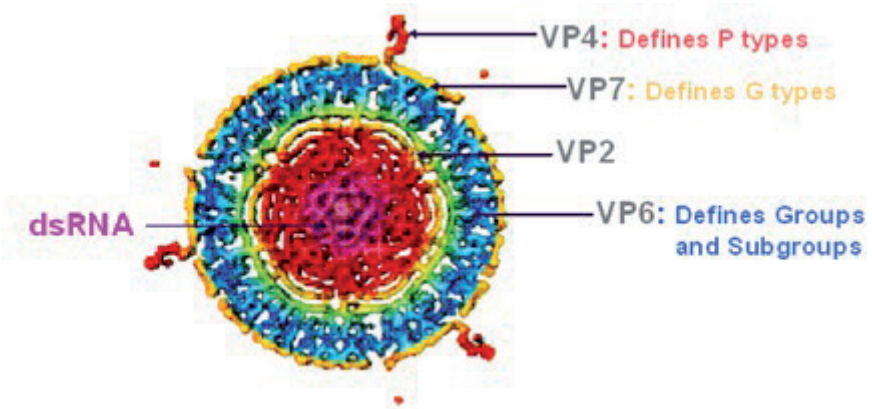

Figure 1. Structure of rotavirus. 2018 @NVMM
Abbreviations: dsRNA = double-stranded RNA, VP = viral protein.

Clinical characteristics

The clinical disease varies from asymptomatic to severe dehydration due to diarrhea, vomiting and fever. Incubation time of rotavirus is short, less than 48 hours. Two mechanisms explain the symptoms. First, rotavirus induces osmotic diarrhea as a consequence of cell damage, necrosis of enterocytes or villus atrophy and as a result of malabsorption. Secondly, NSP4, one of the non-structural proteins, generates secretory diarrhea and activation of the intestinal nerve system.[10] A rotavirus infection leads to fever and general malaise through pro-inflammatory cytokines, interleukins (IL-B and IL-6) and, tumor necrosis factor. The exact mechanism is not yet explained.[10] In addition, rotavirus can cause a systemic infection, rotavirus RNA has been detected in the liver, heart, bladder, lungs, kidneys, testicles and, central nerve system.[15]
Children are usually first infected between four and twenty-three months of age.[9] Presence of symptoms and severe disease course are most frequent during first infection.

Immune response

Immunity following rotavirus infection is primarily achieved through serum and mucosal antibodies against VP7 and VP4. Neutralizing antibodies offer protection against homotypic (against the same virus type) as well as against heterotypic (against a different virus type) rotavirus genotypes. This heterotypic immunity, also named cross-protection, increases with repeated rotavirus infection. The role of cellular immunity is still partly unexplained, however virus specific CD8+ cells potentially clear the infection and protect against disease.[9,16] Rotavirus immunity is highly protective against severe symptoms, however to a lesser extent against infection. Thus, re-infections with rotavirus are common and boost immunity against the virus.[17] A gradual decrease in CD4+ cells and neutralizing antibodies over time can account for incomplete immunity against re-infection.[6,13]

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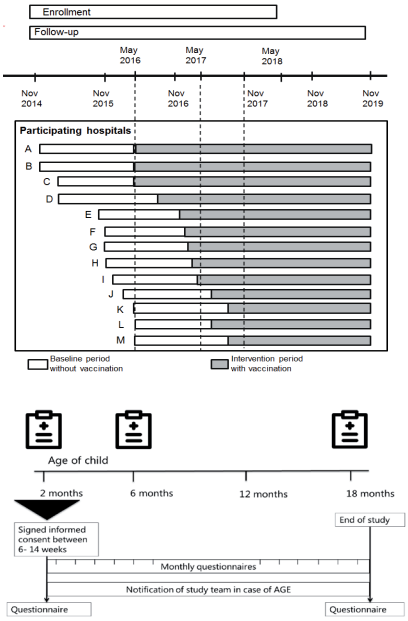
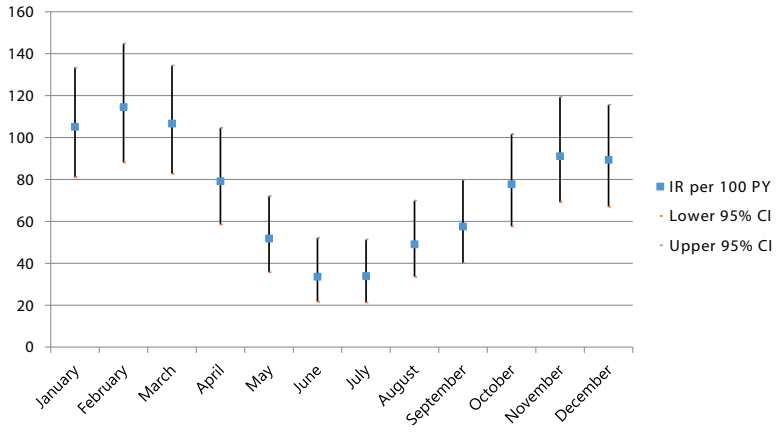


eFigure 2. Schematic outline of study data collection. Abbreviations: Nov = November, AGE = acute gastroenteritis.



eFigure 3. Incidence rate per month. Abbreviations: IR = incidence rate, PY = person years, CI = confidence interval.

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Disease and cost burden	AGE episodes (× 1000)	Hospitalizations[a] (× 1000)	Fatal cases	Vaccine-induced IS	QALYs lost[b]	Life years lost[b]	Net societal costs[b] (mio €)	
Percent reduction	3.4% (2.9–4.0%)	14.7% (13.9–15.3%)	89.8% (86.7–92.2%)	NA	42.7% (23.0–57.7%)	85.6% (72.3–97.2%)	9.4% (8.0–11.0%)	NA
Percent reduction	53.2% (48.1–58.4%)	74.4% (71.9-76.5%)	93.9% (92.7–94.8%)	NA	72.9% (63.0–81.1%)	92.1% (82.5–99.3%)	NA	NA
Universal vaccination vs targeted vaccination[d]								
Absolute change	622 (451–810)	32 (28–36)	4 (1–12)	53.79	769 (561–1003)	90 (9–239)	115 (94–131)	149,282 (101,101 220,113)
Percent reduction	51.5% (46.5–56.7%)	70% (67.4–72.3%)	39.5% (26.9–53.4%)	NA	52.8% (47.6–58.8%)	48.2% (25.4–88.6%)	NA	NA

[a]Including nosocomial infections [b]Using a 3% discount rate for effects (QALYs/life years) and costs

[c]Negative costs are savings [d]Comparing universal vaccination to targeted vaccination in order to obtain the incremental results of extending targeted vaccination to universal vaccination

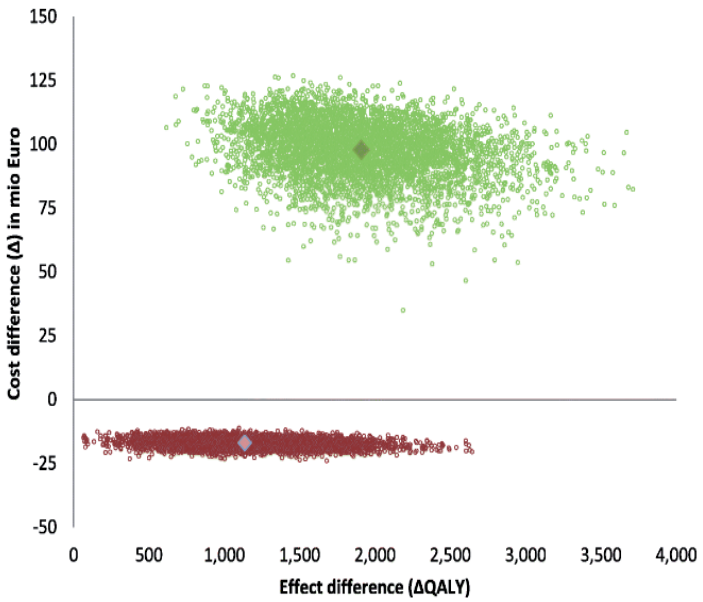

Fig. 3 Cost-effectiveness plane for targeted vaccination (depicted in red) and universal vaccination (depicted in green) using a societal perspective and a 3% discount rate

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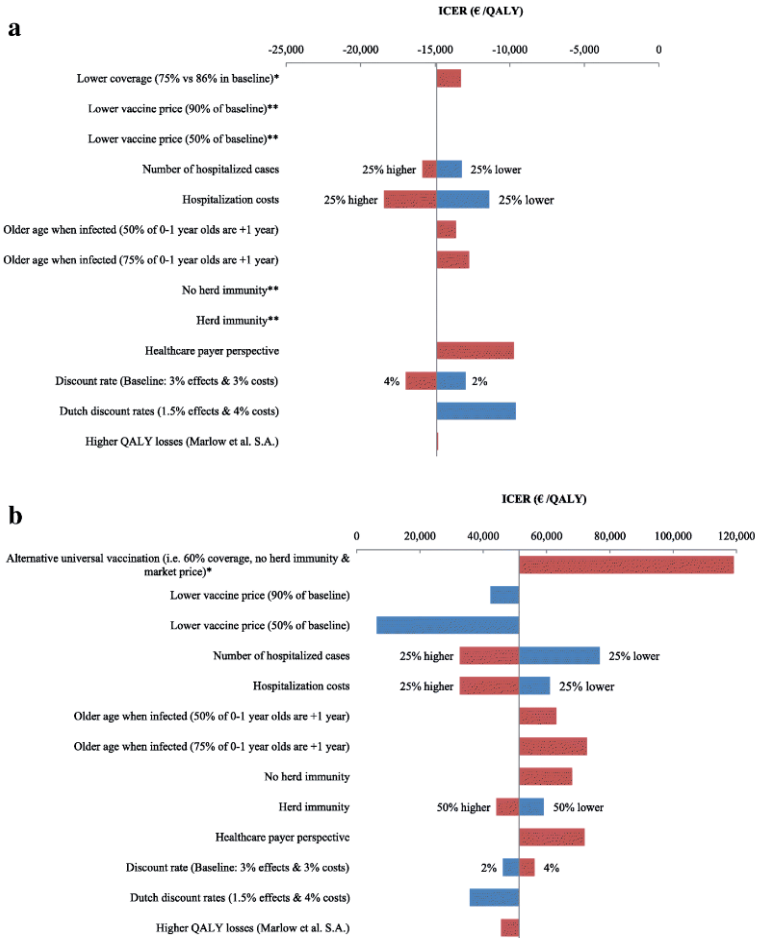

Fig. 4 Tornado diagram showing one-way and multi-way sensitivity and scenario analyses results for a a targeted vaccination strategy and b a universal vaccination strategy

Note1: The x-axis shows the effect of changes in selected variables on the mean incremental cost-effectiveness ratio (ICER) for the base-case taking a societal perspective. The y-axis shows the model parameter that was varied. The bars indicate the mean change in the ICER caused by changes in the value of the indicated variable holding all other parameters similar, whereby a blue bar indicates a lower value of the selected variable(s) as in the baseline and a red bar a higher value of the selected variable(s). Sensitivity analyses with less than 5% changes are not shown. Detailed results are presented in **Table S2** in Additional file 2 for targeted vaccination and in **Table S3** in Additional file 2 for universal vaccination.

Note2: All scenarios for targeted vaccination were cost-saving and health gaining. This results in negative ICERs.

*Some of the sensitivity analyses were only applicable to universal vaccination (i.e. alternative universal vaccination strategy), and others were only to target vaccination (i.e. lower coverage in the target population).

**No S.A. on vaccine price was performed for targeted vaccination as this was already cost-saving at the current market price; No S.A. on herd immunity, as a population vaccine coverage of 7% will not induce herd protection.

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We first compared targeted vaccination to no vaccination over a 20-year time horizon. With annual vaccination costs of €0.64 million, targeted vaccination would avert on average 43,000 rotavirus AGE episodes and 99 fatal cases, and would induce 4.6 IS cases, of which 0.22 would be complicated cases. The targeted vaccination strategy would result in 1139 QALYs gained and €17 million savings (**Table 2**). Targeted vaccination was cost-saving in all simulations (**Fig. 3**) and remained cost-saving in all conducted sensitivity analyses (**Fig. 4a** and Additional file 2: **Table S2**).

We then compared the no vaccination strategy to universal vaccination, which would cost €15 million annually. Over a 20-year time horizon universal vaccination would avert 665,000 rotavirus AGE episodes and 103 fatal cases and would induce 58.4 IS cases, of which 2.8 would be complicated. Universal vaccination would result in 1907 QALYs gained and €98 million additional costs (**Table 2**) at an ICER of €51,280/QALY gained (**Fig. 2** and Additional file 2: **Table S3**). When universal vaccination was compared to targeted vaccination, the ICER increased to €149,280/QALY gained. Sensitivity analyses revealed that vaccine costs, presence and level of herd protection, the perspective chosen (i.e., healthcare costs only vs societal costs), the number of annual rotavirus hospitalizations, the costs per hospitalization, older age at first infection, and productivity losses were most influential on cost-effectiveness results (**Figs. 4, 5** and Additional file 2: **Table S3** and **Figure S1**).

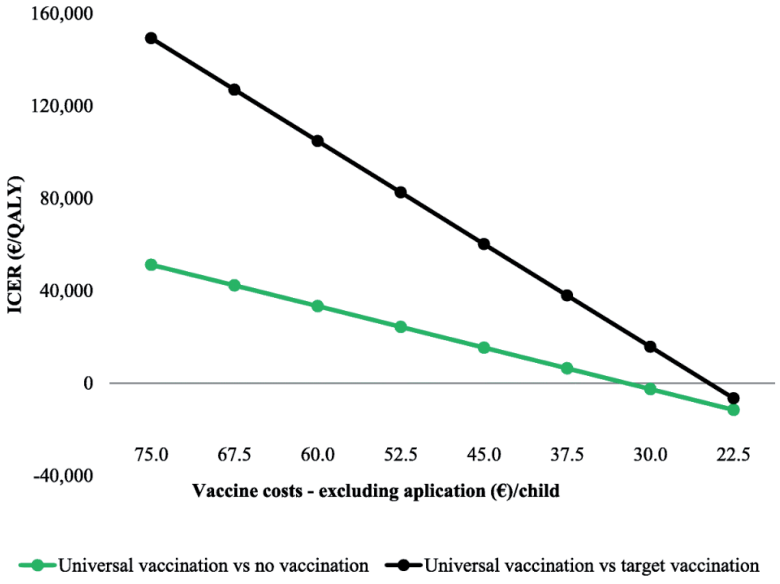

Fig. 5 Mean ICER (cost per QALY gained) for universal vaccination vs no vaccination (green line/dots), and for universal vaccination vs targeted vaccination (black line/dots) using a societal perspective and assuming a discount rate of 3%, for different vaccine costs. Results are also presented in **Table S3** in Additional file 2 (universal vaccination vs no vaccination) and in **Table S4** in Additional file 2 (universal vaccination vs targeted vaccination).

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Scenario	Δ QALY	Δ societal cost (in mio. €)[a]	ICER (€/QALY) - Societal perspective	Δ healthcare cost (in mio. €)	ICER (€/QALY)- Healthcare payer perspective	Induced IS/complicated IS cases	Induced IS: prevented fatal cases	Induced IS: prevented hospitalized cases
	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)
Vaccine costs of €37.5	769 (561-1003)	29 (8-45)	37,978 (9,277-73,241)	63 (54-72)	81,779 (56,779-120,988)	53.8/ 2.6	1:0.09 (1:0.02-1:0.2)	1:597 (1:526-1:668)
Vaccine costs of €30.0	769 (561-1003)	19 (-9-28)	15,717 (cs - 44,867)	46 (37-55)	59,519 (39,427-90,783)	53.8/ 2.6	1:0.09 (1:0.02-1:0.2)	1:597 (1:526-1:668)
Vaccine costs of €22.5	769 (561-1003)	-5 (-26-11)	cost-saving (cs -16,466)	29 (20-37)	37,528 (21,440-61,339)	53.8/ 2.6	1:0.09 (1:0.02-1:0.2)	1:597 (1:526-1:668)
Vaccine costs of €15.0	769 (561-1003)	-22 (-43 - -6)	cost-saving (cs -cs)	12 (2-20)	14,997 (2,738-32,159)	53.8/ 2.6	1:0.09 (1:0.02-1:0.2)	1:597 (1:526-1:668)

Used abbreviations: CI: confidence interval; cs: cost-saving; (cs-cs): 95%CI limits both cost-saving; IS: intussusception.

a)Note: negative costs are savings

Note2: No sensitivity analyses on vaccine costs were applied for the target vaccination strategy, as this scenario was already cost-saving at current market prices of €135.32, for both perspectives. By lowering the vaccine costs, the savings would become larger.

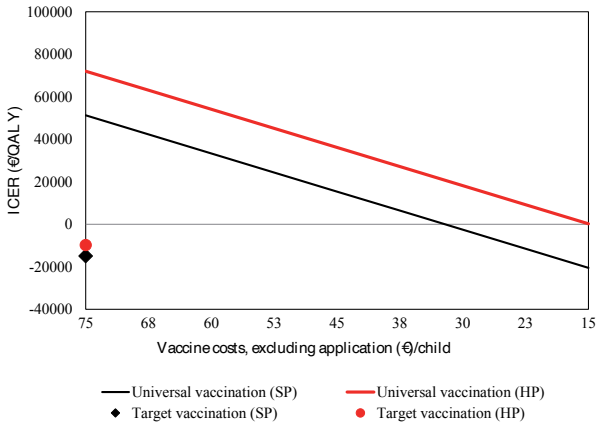

Figure S1: Mean ICER for targeted* and universal vaccination using a societal perspective (black square/black line) and healthcare payer perspective (red dots/red line), for different vaccine costs.

*No sensitivity analyses on vaccine costs were applied for the targeted vaccination strategy, as this scenario was already cost-saving at current market prices of €135.32, for both perspectives. By lowering the vaccine costs, the savings would become larger.

Note: In case of universation vaccination strategy is vaccination cost-saving at vaccine costs of €32/child using a societal perspective (SP) and at €14.8/child using a healthcare payer perspective (HP). For full details see **Table S3**.

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Discussion

In this prospective multicenter study, we evaluated VE and the impact of HRV among 1,482 infants with MRC and prolonged care using the quasi-experimental design of a step-wedge before-after cohort study. We found VE after at least one dose of the HRV to be substantially lower than previously estimated for healthy infants. We were unable to demonstrate statistically significant reductions in any of the pre-specified rotavirus confirmed outcomes. The point estimate for VE against severe rotavirus AGE was 30% in ATP analysis. The IRR for rotavirus AGE of any severity was 1.02 (95%CI 0.69;1.50), suggesting no effect. In subgroup analyses, we observed a trend towards lower VE with lower gestational age, although differences were non-significant.

If protection against rotavirus AGE is desired for these MRC infants, herd immunity (indirect protection via universal vaccination of healthy infants) might be the best alternative. Prevention by indirect effectiveness against rotavirus hospitalizations estimated by meta-analysis (48%, 95%CI 39;55%) was higher than our direct VE estimate.[20]

This low VE after at least one dose of HRV among infants with MRC is unexpected and deserves further discussion. We hypothesize that certain host and pathogen factors could be of influence. For instance prematurity, lower GA is known to be associated with poorer vaccine responses for some, but not all vaccines.[23] In a trial, HRV immune responses in premature infants were found to be of protective levels in 85 ·7% of 147 infants, although this proportion declined with younger GA.[22]

Figure 3. Posterior probability per vaccine effectiveness threshold

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Premature infants in our study were generally of lower GA (33.8% 27-30 weeks compared to 20% in the immunogenicity study), therefore, immaturity of the gut and immune system and consequently a poorer rotavirus vaccine response may explain in part the lower VE in our study. In addition, the gut microbiome, is a known factor of influence on HRV immune response and is different between healthy term and premature infants. Some microbiota species are associated with lower rotavirus vaccine IgA responses,[23] and their presence in premature infants is different than in healthy term infants, depending on gestational maturity.[24] However, we also found low VE in term infants with congenital pathology suggesting that other mechanisms are involved as well. It is important to mention that based on the eligibility criteria, participants in our cohort represent a particularly vulnerable group of infants with prolonged care (between six and 14 weeks of age). For example the median length of post-natal hospital stay was 28 days. In addition, 83.3% of infants in our HRV vaccinated cohort received the recommended two doses and protection is lower after just one dose of HRV in healthy term infants (range 60-92%).[25,26]A pathogen related factor is genotype diversity, protection is primarily against the outer membrane proteins, defining the antigenetically distinct rotavirus G- and P- genotypes.[27] Although HRV elicits both homotypic and heterotypic immunity, the protective effectiveness may differ by rotavirus genotype. A meta-analysis of strain-specific VE found 94%, 87% and, 71% effectiveness against homotypic, fully – and, partially heterotypic rotavirus genotypes, respectively with overlapping confidence intervals.[28] In addition, during our study period (2015-2019) the most dominant circulating strains were all partially heterotypic (G3P8, G4P8 and, G9P8). The homotypic G1P8 rotavirus genotype did not exceed 14% in any of the study years,[29] as was noticed in more high-income countries.[30,31] VE in our study-population may have been influenced by this genotype distribution.

Some limitations need to be addressed. First, fewer than expected rotavirus positive AGE episodes (n=117) were detected, reducing the anticipated statistical power of the study. Cumulative severe rotavirus incidence was assumed at 4% during the 18 months of life for this population, we found only 2.7%. This was however the same for all infants in the Netherlands, the previous expected 3500 annual pediatric rotavirus hospitalizations was adjusted during the study period to 2024.[11,32] This resulted from reduced rotavirus epidemic intensity compared to pre-study years, coinciding with a shift towards a biennial pattern.[33] And furthermore, from incomplete sampling of AGE episodes during follow-up. In a post-hoc analysis we therefore analyzed all-cause AGE as outcome, which is a non-specific, but more sensitive measure of effect. The results were in line with our analysis of the primary outcome. Furthermore, the probability that our study had incorrectly estimated low VE, when true VE would be more than 60% (the estimate used in the sample size calculation) was small.

Second, our study suffered from lower than expected inclusions per hospital and incomplete follow-up. We therefore enrolled five more hospitals, adding to a total of 13 instead of eight, used for sample size calculation. Loss to follow-up occurred in 30% of participants (higher than

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the anticipated ten percent), despite substantial efforts to keep parents of participants engaged in completing questionnaires and reporting AGE. The multiple and complex health issues that many parents of a MRC child encounter during infancy may be barriers to full engagement in an observational study like ours. Mean follow-up however, was at least one year after vaccination (14.5 months). A Cox model takes observation time into account and was adjusted for several covariates, thereby minimizing bias resulting from differential loss to follow-up.

Conclusion

In this population of infants with MRC and prolonged care, administration of at least one dose of HRV implied insignificant protection against severe rotavirus AGE. The findings are in contrast with the general rotavirus VE estimates for healthy infants and underline the importance of conducting separate studies on vaccine performance in specific populations.

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Declaration of Interests: The authors have no financial or personal relationships relevant to this study. None of the authors has any affiliation or received salary from any of the third party funders.

Trial registration: NTR5361 www.trialregister.nl

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Supplementary Material

Methods

Laboratory analyses

Fecal samples were placed in biosecurity envelopes and mailed to the study laboratory for PCR testing. RNA and DNA were isolated from the samples using the MagnaPura96® (Roche Diagnostics, Pleasanton, CA USA) and amplified by ABI75000 realtime PCR system® (Thermofisher Scientific, Foster City, CA, USA). Prior to extraction, a non-human internal control was spiked into the lysis buffer of the samples to monitor for sample inhibition. Positive and negative controls for each pathogen were tested in every run. Fecal samples collected within fourteen days of symptom onset were defined as AGE samples.

Three sources of AGE reporting

- Episodes actively reported by parents with or without a fecal sample collected and including a daily symptom severity score and healthcare attendance. For these episodes we calculated the modified Vesikari Score (MVS). A score of less than eight was defined mild, nine to ten moderate and a score of eleven and more as severe.[1,2]
- Episodes reported on the monthly questionnaire for which the study team was not notified. For these episodes information was available on duration of symptoms and healthcare attendance.
- Episodes retrieved from medical chart review. These included hospitalized episodes only, with or without diagnostic fecal testing performed. Nosocomial AGE was defined as AGE occurring ≥48 hours of admission.

We reported this cohort study according to the STROBE checklist.[3]

Results

Secondary outcomes

Analyses for complete series HRV resulted in an univariate HR of 0.68 (95%CI 0.35;1.34) for rotavirus vaccinated versus unvaccinated infants in ATP cohorts, adjusted for attending daycare and season the HR was 0.62 (95%CI 0.31;1.21). Translating this into a VE of 38% (95%CI -21;69%) after completing the two doses HRV series against severe rotavirus AGE.

The incidence rate (IR) of rotavirus AGE of any severity in the at least one dose HRV vaccinated (ATP) cohort was 67.1 per 1000 person-years (95%CI 51.3;86.1). In the willing to vaccinate (ATP) cohort, the incidence rate was 61·5 per 1000 person-years (95%CI 44.3;83.1). There was no statistical significant difference, incidence rate ratio (IRR) 1.05 (95%CI 0.72;1.55). The negative binomial model resulted in an adjusted IRR of 1.02 (95%CI 0.69;1.50), corresponding with a VE of -2% (95%CI -50;31%).

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Overall, there was little difference in results per pre-specified subgroup compared to the full cohorts, but confidence intervals were wider because of smaller numbers. The estimated VE after at least one dose against severe rotavirus AGE varied between 3.3% for the subgroup of infants with a gestational age 30-32 weeks and 49.0% for subgroup of term infants with congenital disorders. See **table S1**.

Table S1. Coefficients and hazard rates for subgroups

Subgroup	Coefficient*	95% CI	Multivariate Hazard ratio*
Gestational age 32-37 weeks	-0.12	-2.08;0.37	0.89
Gestational age 30-32 weeks	-0.03	-1.45;2.80	0.97
Gestational age below 30 weeks	-0.26	-1.74;1.53	0.77
Term and congenital disorder	-0.67	-3.30;5.50	0.51
Multiple risk conditions	0.25	-1.41;4.79	1.28

Rotavirus severe AGE as outcome comparing vaccinated versus unvaccinated infants in ATP cohorts using the main analysis Cox model with an interaction term for subgroup. *Adjusted for daycare attendance and rotavirus epidemic intensity. Abbreviations: CI = confidence interval.

All-cause AGE subgroup analyses

With all-cause severe AGE as outcome in subgroup analyses, premature infants (GA 32-37 weeks) and term infants with a congenital disorder had a higher estimated VE (44.6% and 68.0% respectively). And preterm infants of lowest gestational age (<30 weeks) or infants with multiple conditions seemed to benefit less from HRV vaccination (VE -3.6% and 4.4% respectively).

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CONGENITAL DISORDERS ACCORDING TO ICD-10	Qualifies as eligible		
	Yes	No	Sometimes
Congenital malformation central nervous system and senses			
Anencephaly			
Microcephaly			
Spina bifida and meningo(myeleo)cele			
Encephalocele			
Neuromuscular disease			
Hydrocephalus/holoprosencephaly without neural tube defect			
Other congenital CNS malformation			
Congenital defect in senses			
Microphthalmia			
Other congenital eye disorders			
Inborn errors of the ears			
Other innate sense abnormalities			
Congenital anomaly cardiovascular			
Lack of umbilical cord artery			
Transposition of the large vessels			
Tetralogy of Fallot			
Ventricle septum defect			
Hypoplastic left heart syndrome			
Coarctation of the aorta			
Tricuspidis atresia / stenosis			
Complicated heart defect			
Other birth defects of heart and blood vessels			
Congenital anomaly digestive system			
Cleft lip with or without cleft palate			
Split palate without cleft lip			
Esophagus atresia/stenosis/fistula			
Intestinal/anal atresia			
Hirschsprungs' disease			
Malrotation/volvulus			
Other congenital disorder of digestive tract			
Congenital respiratory abnormality			
Choanal atresia			
Tracheal disorder			
Lung hypoplasia			
Lobar emphysema			
Hydro/chylo thorax			
Diaphragmatic hernia			
Relaxation of diaphragm			
Other congenital respiratory disorders			
Congenital malformation urogenital system			
Hypospadias and/or epispadias			

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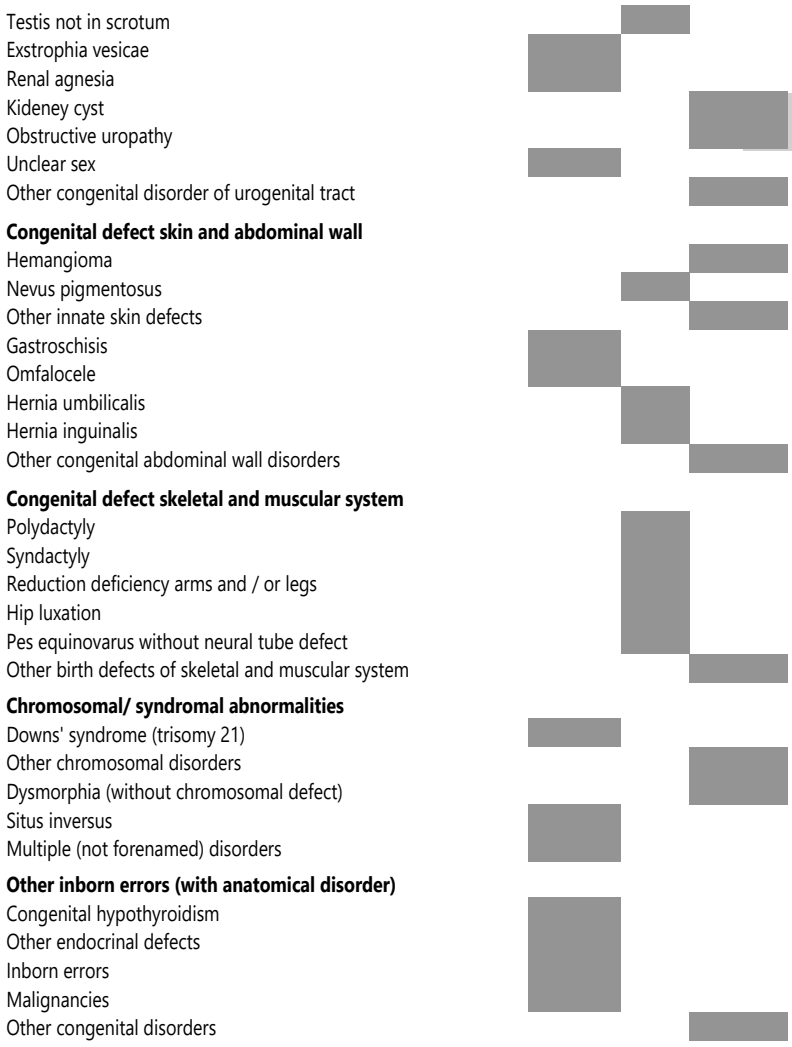

*Complex Chronic Condition: (1) are expected to last longer than 12 months (2) involve either several different organ systems or 1 organ system severely enough to require specialty pediatric care and hospitalization at some point.

Figure S1. List of eligible congenital disorders[4]. Abbreviations: ICD-10 = international code of diseases 10th edition. *Qualifying conditions are those that last longer than 12 months, involve multiple organ systems and/or are expected to require pediatric specialty care. Defined into categories: A. Cardiovascular, B. Pulmonary, C. Neurodevelopmental, D. Chromosomal, E. Perinatal and F. Other.

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Table S2. Characteristics of infants with severe rotavirus AGE among HRV vaccinated and unvaccinated infants

Case	Sex	Gestation in week + days	Birthweight in grams	Congenital disorder	SGA	HRV	Age* severe rotavirus AGE	Age* HRV1	Age* HRV2
1	M	34+6	1800	no	yes	yes	10	2	
2	M	37+0	2100	no	yes	yes	14	1	3
3	M	25+2	900	no	no	no	7		
4	M	36+1	2500	yes	no	yes	11	1	
5	M	37+2	3500	yes	no	yes	3	2	4
6	F	41+0	3500	yes	no	yes	15	2	3
7	M	29+4	1300	no	no	no	8		
8	F	35+6	2500	no	no	no	16		
9	F	29+0	1200	no	no	yes	10	1	3
10	F	33+2	2000	no	no	no	4		
11	F	32+2	2200	no	no	yes	2	1	
12	F	32+0	1800	no	no	yes	14	1	4
13	M	35+0	3000	no	no	yes	5	1	4
14	M	32+4	1800	no	no	no	12		
15	F	30+6	1600	no	no	no	9		
16	M	31+4	1300	no	yes	yes	13	1	
17	M	31+6	1800	no	no	yes	12	1	4
18	M	30+1	1600	no	no	no	12		
19	F	27+5	1000	no	no	yes	17	1	3
20	M	31+2	1700	no	no	no	9		
21	F	29+2	1300	no	no	no	15		
22	M	35+6	1900	no	yes	no	13		
23	M	36+0	2200	no	yes	yes	10	2	3
24	M	31+0	2000	no	no	yes	9	1	4
25	F	30+3	1600	no	no	yes	13	1	2
26	M	35+0	2100	no	yes	no	9		
27	M	36+0	2200	no	yes	no	15		
28	F	25+6	800	yes	no	no	15		
29	F	25+6	700	yes	yes	no	15		
30	M	35+1	3700	no	no	no	10		
31	F	38+5	3200	yes	no	no	5		
32	M	35+6	3100	no	no	no	13		

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Case	Sex	Gestation in week + days	Birthweight in grams	Congenital disorder	SGA	HRV	Age* severe rotavirus AGE	Age* HRV1	Age* HRV2
33	F	41+3	3000	yes	yes	yes	5	3	4
34	F	34+0	1700	yes	yes	yes	15	2	

* Age in months. Abbreviations: SGA = small for gestational age, AGE = acute gastroenteritis, HRV = human rotavirus vaccination, HRV1 = first dose of human rotavirus vaccination, HRV2 = second dose of human rotavirus vaccination.

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Chapter 5

Safety and Tolerability of Human Rotavirus Vaccination in Medical Risk Infants in the Netherlands

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Under review, revisions submitted

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Abstract

Objective: Rotavirus vaccination is recommended for prevention of severe rotavirus disease. While the vaccine is well tolerated in healthy term infants, there are limited data on premature infants. We aim to assess the safety and tolerability among infants born between 27-37 weeks of gestation in need of prolonged postnatal medical care.

Methods: The Netherlands has no national rotavirus immunization program, but selective human rotavirus vaccination (HRV) for high-risk premature infants was implemented in participating hospitals. HRV vaccination and occurrence of any vaccine related serious adverse event (SAE) was systematically documented. As secondary endpoints solicited adverse events (AE) following administration of national immunization program (NIP) vaccines with or without HRV were prospectively collected.

Results: Among 2077 rotavirus vaccinated infants, ten vaccine related SAE were documented, resulting in hospital admission or prolonged hospitalization. There were no deaths and all infants recovered. The adjusted risk ratio (RR) for any AE following concomitant NIP+HRV administration was 1.07 (95%CI 1.04-1,10) and, for HRV administration alone RR was 0.90 (95% CI 0,80-0.98), compared to NIP administration. Gastrointestinal AE were statistically significant reported more often after receiving HRV (p-value <0,001).

Conclusion: Among vulnerable premature infants, vaccine related SAE were reported in 0.25 per 100 HRV vaccine doses. HRV is generally well tolerated when co-administered with NIP vaccines, but associated with approximately ten percent higher risk of gastrointestinal AE.

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Introduction

Premature infants are at increased risk of severe rotavirus acute gastroenteritis.[1–4] Therefore prevention of rotavirus disease is particularly important for premature infants. Since 2006 two rotavirus vaccines have been licensed; RotaTeq© (Merck and Co, USA) and, Rotarix© (GSK, Belgium). Both vaccines were previously studied in premature infants and demonstrated efficacy and immunogenicity.[5,6] Findings on rotavirus vaccine safety and tolerability from these studies indicate that rates of adverse events (AE) are similar to those observed among term infants.[6,7] However the trials included a limited number of infants with gestational ages (GA) less than 30 weeks, and for Rotarix (GSK), no infants with GA < 27 weeks were included. Moreover, inclusion criteria required premature infants were healthy and medically stable at time of enrollment. This relatively healthy study population may not be representative, as a complicated postnatal course, is common in (very) premature infants, and may influence AE rates.[6,7]

Very premature infants (GA <30 weeks) can experience more and different vaccine AE following routine vaccines other than rotavirus vaccination. A recent review found that cardiorespiratory events (apnea, bradycardia and desaturation) occurred in 13-30% of very premature infants following DTaP-IPV-Hib-HepB vaccine.[8] In addition vomiting and hypotonia occurred more frequently in premature infants, while rates of local and other general symptoms were comparable.[8]

More real-world data on safety and tolerability of rotavirus vaccination in these infants, with or without concurrent administration of other routine immunizations, is needed to guide clinicians in the individual assessment of risks and benefits and to counsel parents on anticipated AE.

In the Netherlands, a national rotavirus vaccination policy has not been introduced yet, but a pilot was conducted in 13 Dutch hospitals implementing rotavirus vaccination in routine neonatal care for medical risk infants, including premature infants (Risk-group Infant Vaccination Against Rotavirus [RIVAR] project). This selective vaccine strategy was supposed to prevent those infants most at risk of severe disease.[2] Within this setting we evaluated the safety and tolerability of rotavirus vaccination among medical risk infants. Here we focus on premature infants with gestational ages between 27-37 weeks. Because infants with GA below 27 weeks are vaccinated off-label, and the decision to offer HRV was based on local policy, the group was analysed and described separately. Data on infants with other medical risk conditions are described in supplementary material.

Patients and methods

Study setting and subjects

For a full description of the RIVAR project we refer to **chapter 4** and the Dutch trial registry.[9] In brief, an implementation project for selective rotavirus vaccination was conducted in

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participating secondary pediatric hospitals. Eligible infants included those with prematurity, low birth weight and/or, presence of a severe congenital disorder (list added in **chapter 4**). Eligibility further required an inpatient hospital stay or a planned outpatient visit between six and 14 weeks postnatal age, allowing for first dose rotavirus vaccine administration on site within the appropriate age-window. The target population therefore represented infants with medical risk conditions who required prolonged pediatric care after birth. Within participating hospitals, a prospective cohort study was set-up starting enrollment from one year before rotavirus vaccination implementation (rotavirus unvaccinated infants), until 12-18 months post-implementation (rotavirus vaccinated infants). Participating infants were followed from approximately six weeks until 18 months of age for occurrence of acute gastroenteritis to evaluate vaccine effectiveness as primary outcome. In addition, all vaccinations received and solicited AE were prospectively documented for tolerability assessment as secondary outcome. To guide recommendations of rotavirus vaccination, tolerability was analyzed for subgroups of infants with medical risk conditions separately.

Data collection

Safety assessment was a secondary outcome of the RIVAR project. The research staff reviewed medical records for each rotavirus vaccination eligible infant at approximately five months of age to check rotavirus vaccination dates and documentation of any vaccine related serious vaccine adverse reaction (SAE) following administration. Vaccine related SAE was defined as any reaction that was fatal, life threatening, disabling or incapacitating, required in-patient treatment or prolonged existing hospitalization, or which required intervention to prevent the previously stated outcomes and, considered related to rotavirus vaccination as judged by the treating physician and documented in the patient medical record (**Figure 1**). All eligible infants with at least one HRV administration were included in the analysis. In addition, we described "vaccine failure" when rotavirus acute gastroenteritis led to hospitalization and occurred at least 14 days after second HRV dose.

For tolerability assessment, we used data from participants of the before-after cohort study. Monthly parental questionnaires contained date and type of the NIP primary series and/or HRV immunizations received, the occurrence of solicited AE and, whether they sought healthcare, in the seven days following administration. Solicited AE were fever, rash, irritability, loss of appetite, vomiting, looser and/or, bloody stools. We defined vaccine administration concomitant when vaccination dates were identical for at least one NIP and HRV (NIP+HRV) vaccination or if they were with a maximum of three days apart, such that the seven-day post-vaccination period for reporting solicited AE covered both vaccinations. All monthly data from cohort participants that included information on type and date of vaccination and AE reporting were included in the analysis. The standard vaccination schedule is shown in **Figure 1**.

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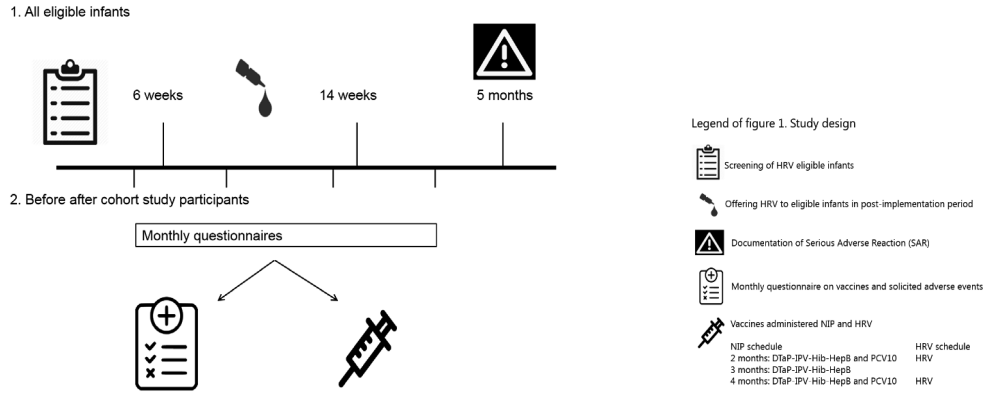

Figure 1. Study design and primary series NIP schedule. Abbreviations: HRV = human rotavirus vaccine, NIP = national immunization program, DTaP-IPV-Hib-HepB = hexavalent diphtheria-tetanus toxoids-accelular pertussis-inactivated polio-Haemophilus influenzae type B-hepatitis B vaccine, PCV10 = 10-valent pneumococcal vaccine.

Outcomes

1) The outcome for safety was defined as the number of vaccine related SAE per 100 vaccine doses in high-risk premature infants.

2) For tolerability, we compared the occurrence of at least one solicited AE in the seven days following receipt of either NIP vaccines (DTaP-IPV-Hib-HepB and/or PCV10), concomitant NIP+HRV or, HRV only. We estimated the relative risk of AE per vaccine administration with NIP only as reference. The number of solicited AE per vaccine administration was compared using incidence rate ratio (IRR). Comparisons were performed for the subgroup of fever and gastrointestinal AE (vomiting, bloody and/or, looser stools) and, for AE related healthcare attendance.

In secondary analysis of tolerability we reported the cumulative incidence of solicited AE that infants experienced during completion of the primary series of either NIP or NIP+HRV. In addition, occurrence of fever, gastrointestinal solicited AE and, AE related healthcare attendance were described.

Analysis

1) Each vaccine related SAE was described in terms of diagnosis, infant characteristics, timing in relation to HRV vaccination and, where applicable, results of faecal testing. Rotavirus positive faecal samples were additionally genotyped, RNA was extracted from faecal samples using the MagnaPure96 nucleic acid extraction system. The purified RNA was subsequently subjected to PCR amplification and sequencing of the VP4 and VP7 genes according to Simmonds

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et al. and Zeller et al., respectively.[10,11] The obtained sequences were used for rotavirus type determination in the web-based typing tool RotaC.[12]

2) Comparison of baseline characteristics between the groups was done using Chi-square, student-T or non-parametric tests depending on the distribution. For the primary tolerability outcome of at least one AE we used a mixed model with binomial distribution and a random intercept per infant. We estimated odds ratios for type of vaccine administration (NIP, HRV or NIP+HRV), adjusting for age at vaccination. Odds ratio was transformed into risk ratio (RR) using the method described by Knol et al.[13] Predefined covariates in multivariate analysis included: GA, small for gestational age, presence of congenital disorders, older siblings, parental age, parental background and, socio-economic status. To assess whether the effect of co-administration of HRV on solicited AE was dependent on GA, we added an interaction term to the model. The final model was selected based on the Akaike Information Criterion. For the number of solicited AE experienced after vaccine administration, we estimated adjusted incidence rate ratio (IRR) using negative binomial regression. We performed complete case analysis, potentially induced bias was explored by comparing complete cases to those with missing information. As statistical software SPSS version 25.0.0.2 and RStudio version 3.6.1 were used, with packages *lme4, MASS*.

Results

1) Safety

A total of 5730 high-risk premature infants ≥ 27 weeks of GA fulfilled the eligibility criteria, figure 2. The median GA was 33 weeks and 1 days, 1962 (34.2%) were small for gestational age and, 549 (9.6%) had one or more congenital pathologies. Among 2077 HRV vaccinated infants ten vaccine related SAE were documented, 0.25 per 100 vaccine doses. These included two cases of intussusception (one ultrasound confirmed), two cases of necrotizing enterocolitis and, one clinical sepsis (no pathogen detected). One infant developed acute gastroenteritis that required hospital admission (shortly after vaccination, no stool sample available) and one infant lactose intolerance (occurrence of diarrhea and severe abdominal cramps after both vaccine doses, which resolved after lactose free formula milk was introduced). Three infants developed sudden cardiorespiratory events. For detailed information see Appendix A **table S1.** All infants recovered and there were no deaths. Two infants with hospitalized rotavirus acute gastroenteritis were classified as vaccine failures, partially heterotypic genotypes were detected in sampled feces. In particular regarding the VP4 genotype, which in both cases did not match the genotype of the vaccine strain.

Five out of 13 hospitals had a policy to vaccinate infants with GA < 27 weeks, 82 out of 92 (85.9%) eligible infants were vaccinated with at least one dose of HRV. No vaccine related SAE were documented. For safety data concerning term infants with congenital disorders, see **appendix B**, among 118 vaccinated infants two vaccine related SAE were documented.

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2) Tolerability

The group of high-risk participating premature infants ≥ 27 weeks of GA consisted of 1206 infants. Data on at least one vaccine administration and AE were available for 1041 (86.3%) infants, including 471 in the NIP only and 570 in the NIP+HRV group (**figure 2**). Patient characteristics of NIP versus NIP +HRV vaccinated infants were largely comparable (**table 1**). Age at first vaccination was slightly younger among HRV vaccinated infants (59 days versus 61 days of postnatal age, p=0.0). In the seven days following 1350 vaccinations any AE was reported out of 3031 reported vaccine administrations (44.5%). The total number of reported AE was 2057, resulting in an incidence rate of 0.68 (95%CI 0.65;0.71) per vaccine administration. A total of 134 vaccine administrations were followed by an AE related healthcare contact (4.4%).

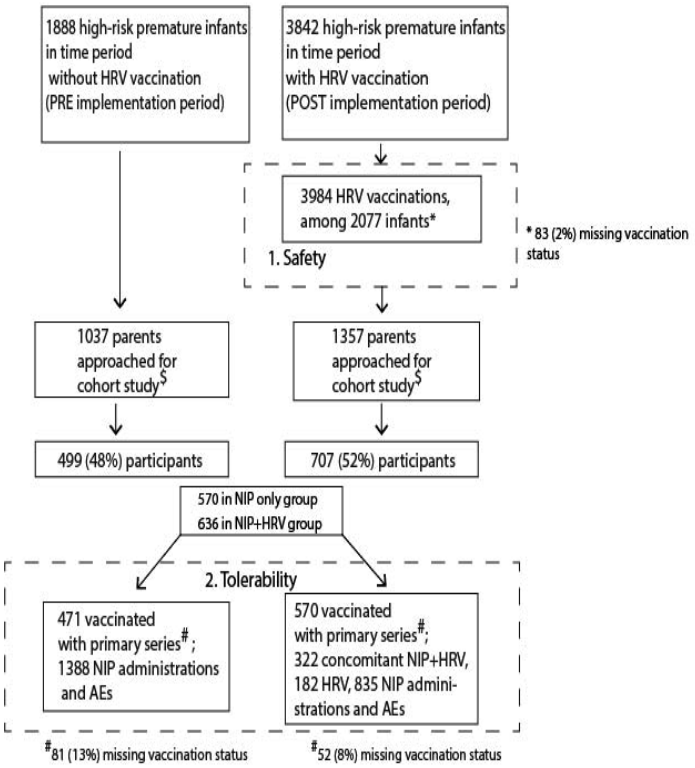

Figure 2. Flowchart of high-risk premature infants and cohort study-participants with gestational age between 27 and 37 weeks. $Reasons for not approaching eligible infants: 446 versus 484 discharged or referred, 154 versus 124 language barrier, 147 versus 80 unstable social environment, 38 versus 41 poor prognosis, 96 versus 130 other unspecified reason, 14 missing and 1626 eligible during follow-up, when recruitment for cohort study was ended. Abbreviations: HRV = human rotavirus vaccine, NIP = national immunization program, AE = solicited adverse event.

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Table 1. Baseline table of vaccinated infants with gestational age ≥ 27 weeks

Characteristic	NIP vaccinated (N=471)	NIP + HRV vaccinated (N=570)	p-value
Gestational age in weeks+days (mean, SD)	32+1 (2+4)	32+3 (2+4)	0.07
Gestation subgroups			0.16
27-30 weeks	113 (24.0)	116 (20.4)	
30-37 weeks	358 (76.0)	454 (79.6)	
Birthweight in grams (median, IQR)	1770 (840-2700)	1780 (990-2570)	0.80
Small for gestational age (yes,%)[£]	135 (28.7)	180 (31.6)	0.31
Sex (male, %)	269 (57.1)	301 (52.8)	0.17
Multiple birth (yes, %)	122 (25.9)	157 (27.2)	0.64
Congenital pathology (yes, %)	38 (8.1)	48 (8.4)	0.84
Number of disorders			0.49
1 (%)	30 (78.9)	42 (87.5)	
2 (%)	5 (13.1)	6 (12.5)	
>2	3 (7.9)	-	
Age at first vaccination in days (median, IQR)	61 (48-74)	59 (40-78)	**<0.001***
Sibling (yes, %)	99 (21.5)	120 (21.4)	0.97
Parental education[#]			0.44
High	342 (73.5)	422 (75.4)	
Medium	104 (22.4)	123 (22.0)	
Lower	19 (4.1)	15 (2.7)	
Parental background[^]			0.85
European	399 (85.6)	477 (85.0)	
Non-European	25 (5.4)	28 (5.0)	
Mixed	42 (9.0)	56 (10.0)	
Average parental age in years (median, IQR)	32.5 (27.0-38.0)	33.0 (27.0-39.0)	0.41

Percentages are derived excluding subjects with missing data on the variable. Statistical significance (p-value <0.05) is highlighted in bold. *Rank-sum test. [£] Based on 10th percentile perinatal growth curves.[14] #Based on highest parental educational level. ^Based on parental background and categorized according to world population by country.[15] Abbreviations: HRV = human rotavirus vaccine, N = number in group, SD = standard deviation, IQR = interquartile range.

Overall, there was a 10% difference in occurrence of any solicited AE following NIP (45.5%) versus NIP+HRV (55.7%) vaccine administration, **table 2**. AE were reported less frequently for HRV only administration (36.4%), largely due to a reduced frequency of fever (5.2%). Gastrointestinal AE were more frequent after HRV only administration (16.4%) and after concomitant NIP+HRV administration (17.0%) compared to NIP vaccination (6.0%). No

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differences were observed in the frequency of related healthcare attendance between NIP only, concomitant NIP+HRV or, HRV only administrations.

The adjusted RR for at least one solicited AE in the seven-day post-vaccination period was 1.07 (95%CI 1.04-1.10) for concomitant NIP+HRV versus NIP only and 0.90 (95%CI 0.80-0.98) for HRV only vaccination (**table 3**). The final model included GA, presence of a sibling in the household and, age at vaccination as covariates. The interaction term for GA was not statistically significant. Analysis of the number of AE per vaccine administration yielded comparable results with an adjusted IRR of 1.07 (95% CI 0.96-1.19) for NIP+HRV versus NIP only (**table 3**). GA, sibling and, age at vaccination were included in the model.

Table 2. Solicited AE following receipt of NIP vaccines, concomitant NIP+HRV or HRV only vaccination as part of the primary series in high-risk premature infants (≥ 27 weeks of gestational age)

Reported adverse events	NIP only vaccination N=1233	Concomitant NIP +HRV vaccination N=305	HRV only vaccination N=176	p-value
At least one solicited adverse event	558 (45.5%)	170 (55.7%)	63 (36.4%)	**<0.00**
Fever	213 (17.3%)	60 (19.7%)	9 (5.2%)	**<0.00**
Gastrointestinal	74 (6.0%)	50 (16.4%)	30 (17.0%)	**<0.00**
Mean number of solicited adverse events	1.49 (SD 0.81)	1.55 (SD 0.76)	1.79 (SD 0.97)	**<0.00***
Number of solicited AE				0.08
1	363 (29.6%)	101 (33.1%)	31 (17.9%)	
2	139 (11.3%)	47 (15.4%)	20 (11.6%)	
>2	56 (4.6%)	22 (7.2%)	12 (7.0%)	
Any AE related healthcare attendance	59 (4.8%)	15 (4.9%)	7 (4.0%)	0.78#
Type of healthcare				0.18#
General practitioner	18 (1.5%)	2 (0.7%)	4 (2.3%)	
Outpatient clinic	7 (0.6%)	2 (0.7%)	1 (0.6%)	
Emergency room	12 (1.0%)	-	1 (0.6%)	
Hospitalization	32 (2.6%)	12 (3.9%)	3 (1.7%)	

Statistical significance (p-value <0.05) is highlighted in bold. *ANOVA test. # Fisher Exact test. Abbreviations: HRV = human rotavirus vaccine, N = number in group, NIP = national immunization program.

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Table 3. Outcome estimates

	Unadjusted estimate	95% CI	Adjusted estimate*	95% CI
NIP only vaccination	Ref	-	Ref	-
At least one solicited AE after concomitant NIP+HRV vaccination	RR 1.07	1.03-1.10	RR 1.07	1.04-1.10
At least one solicited AE after HRV only vaccination	RR 0.90	0.79-0.98	RR 0.90	0.80-0.98
Number of solicited AE after NIP+HRV vaccination	IRR 1.06	0.96-1.18	IRR 1.07	0.96-1.19

*Adjusted for age at vaccination (in months), older sibling in household, gestational age in weeks. Abbreviations: RR = relative risk, IRR = incidence rate ratio CI = confidence interval, NIP = national immunization program, HRV = human rotavirus vaccine.

Results of secondary outcomes are presented in appendix A, **table S2**. A total of 736 infants (70.7%) reported any solicited AE in the seven-day post-vaccination period during completion of the primary series with or without HRV and 99 (9.5%) infants had any AE related healthcare contact.

Characteristics for infants with GA <27 weeks were relatively similar with the exception of older age at vaccination for HRV vaccinated infants (72 versus 63 days), shown in **table S3** of Appendix A. Tolerability for infants with a GA below 27 weeks is different than for those born past 27 weeks of gestation, fewer AE were reported, however numbers are small (see appendix A **table S3 and S4**).

The tolerability among 134 term infants with congenital disorders was similar as among high-risk premature infants of ≥ 27 weeks GA and is described in **appendix B**. Exploration of potential bias induced by complete case analyses are shown in appendix A, **table S5**. There were statistical significant less infants born as twins among those with missing information, and average parental age was lower as was family educational level.

Discussion

Our findings indicate that about one in 200 high-risk premature infants experienced a vaccine related SAE following a two-dose HRV course that was associated with hospitalization, increased length of hospital stay or life support intervention, but all resolved without long-term sequelae. Administration of HRV with or without concomitant administration of NIP vaccines was

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generally well tolerated, although risk of AE increased by seven percent when HRV was added. Gastrointestinal AE were reported in one in four infants who received HRV. Reassuringly, this increased rate of (gastrointestinal) AE did not result in more frequent healthcare attendance for AE among NIP versus NIP+HRV vaccinated infants, ten versus nine percent, respectively. For HRV only vaccine administration fever as AE is less commonly reported (5.2%) compared to NIP vaccines (17.3%).

Rotavirus vaccines have been available worldwide since 2006, and over 100 countries have implemented a globally licensed vaccine in their infant immunization program.[16] In Cochrane systematic reviews on safety of these vaccines, SAE were reported in 2.3-7.6% of healthy term infants following vaccination, no increased risk compared to placebo.[17,18] Gastrointestinal SAE occur in 0.1-0.6% of HRV vaccinated term infants, but this is without mention of possible relatedness to vaccination.[19–21] A prior study, among infants generally healthy and on average of older gestational age, identified two vaccine related SAE among 670 HRV vaccinated premature infants,[6] which is lower than in our study. Given the clinical vulnerability of the infants in our study receiving rotavirus vaccination, an active approach and systematic screening of all medical records was chosen to guarantee complete case finding. However, it makes it difficult to interpret our finding of 0.25 vaccine related SAE per 100 vaccine doses in the context of existing evidence. It is well known that rates of (vaccine related) SAE following vaccination are generally higher in premature infants,[8] but to what extend this also applies to HRV is uncertain. Our findings indicate that clinicians should be vigilant about the possibility of vaccine related SAE when vaccinating against rotavirus in infants with prematurity and clinical vulnerability.

In line with results of prior HRV tolerability studies in premature and term infants,[6,19–21] HRV seemed well tolerated among high-risk premature infants and was associated with only a small increase in AE when combined with NIP vaccines. The observed frequency of AEs in 45-55% of our study population is even slightly lower than the 60% reported in a Dutch study of healthy term infants receiving NIP vaccines.[22] Gastrointestinal AE appear a class effect of oral rotavirus vaccines and occur in up to 25% of premature infants in our and other studies, but they rarely require medical attention.[6,7,23] Although symptoms may be mild, it is important to counsel parents about this possible side effect prior to vaccination.

Because infants with GA < 27 weeks were excluded from the pre-licensure trials of HRV, administration of HRV to these infants is off-label. In our study, fewer solicited AE among infants of GA < 27 weeks were reported to those among premature infants ≥ 27 weeks. No vaccine related SAE was documented. Although the numbers are small, these findings are reassuring.

The Netherlands currently has no national rotavirus vaccine policy and uptake in private market is very low.[24,25] We recently showed that vaccine effectiveness of at least one dose HRV among our study population of medical risk infants was substantially lower than expected at 30%

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(**chapter 4**). This raises the question whether the benefits of rotavirus vaccination outweigh the possible risks of vaccine related SAE in this particular population, especially in a setting with low rotavirus incidence as currently seen in the Netherlands.[26,27] Alternatively, protecting these infants through herd immunity by implementing universal infant vaccination against rotavirus may be a strategy worth considering. It has been shown that herd immunity effects can reduce the risk of severe rotavirus acute gastroenteritis by 48% among unvaccinated infants.[28]

Important limitations should be mentioned. First, for the observed vaccine related SAE we cannot confirm or exclude causality between the event and administration of HRV. In our evaluation, we relied on clinical judgement by the treating physician, considering timing of the event in relation to vaccine administration and/or type of SAE and patient comorbidities. At most, we can therefore conclude that a causal link is plausible. For some vaccine related SAE, other vaccines co-administered with HRV may also have triggered the event.

Second, our results on tolerability assessment are based on parent reported solicited AE, which may be subject to variability in perception between parents. Although fever, vomiting and loose stools can be assessed quite objectively, we cannot rule out that reporting may have been influenced by whether infants received the newly introduced HRV, which could increase parent's attentiveness to AE. This effect is likely small as percentages of parent reported AE between the two groups were similar. Differences were only observed for gastrointestinal adverse events, which is in line with previous results on tolerability of HRV.

Third, data were missing on some covariates and vaccinations in approximately 10% of infants. Our analyses are based on complete cases only, by comparison of infants with missing versus complete data, we explored the potential of bias this induced. Although family educational level was lower among infants with missing data, educational level was not associated with AE in univariate analysis and therefore complete case analysis is justified and results are generalizable to the broader population of vulnerable premature infants.

Conclusion

In conclusion, we observed higher incidence of vaccine related SAE following HRV among vulnerable premature infants than reported in literature. Clinicians should be aware and must outweigh the risks and benefits of HRV for this particular group. HRV administration is generally well tolerated, also in infants < 27 weeks of GA, but associated with approximately ten percent higher risk of gastrointestinal AE when co-administered with NIP vaccines.

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Supplementary Material

Appendix A

Table S1. Detailed information on vaccine related SAE and hospitalized vaccine failure after first and second HRV administration

Nr	Event	Sex	Gestational age in weeks + days	Birth weight in grams	Congenital disorder	Age at HRV1*	Age at HRV2*	Age at SAE*	Rotavirus positive feces sample
2	Severe AGE	M	32+3	2000	no	52		55	
3	Intussusception	F	33+5	1500	no	49	120	124	
4	Cardio-resp incidents	F	30+3	1300	no	50	80	51	
5	Sepsis	F	28+4	800	no	56	91	92	
6	NEC	M	27+4	1000	no	58		62	no
7	Lactose intolerance	M	32+0	1600	no	68	120	120	
8	NEC	M	33+1	2000	yes	68		68	yes
10	Intussusception	F	33+0	1500	no	95	126	127	
11	Cardio-resp incidents	F	28+3	700	no	58	108	109	
13	Cardio-resp incidents	M	27+3	2900	yes	99		99	
16	Vaccine Failure	M	27+2	800	no	109	144	344	yes (Genotype G3P8)
17	Vaccine Failure	M	31+6	1800	no	51	125	384	yes (Genotype G9P8)

* Age in days. Abbreviations: M=male, F=female, SAE = serious adverse event, HRV = human rotavirus vaccine, AGE = acute gastroenteritis, Cardio-resp = cardiorespiratory events, NEC=necrotizing enterocolitis, HRV1 = first dose, HRV2 = second dose.

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Secondary outcomes

Results of the secondary endpoints are presented in **table S2**. The mean cumulative number of solicited AE during the primary series of NIP was 1.77 compared to 1.97 for NIP+HRV vaccinated infants.

Table S2. Solicited AE for receipt of the primary series with or without HRV per infant (≥ 27 weeks of gestational age)

Reported adverse events associated with receipt of primary series	NIP vaccinated infants (N=471)	NIP+HRV vaccinated infants (N=570)	p-value
Any solicited adverse event	332 (70.5%)	404 (70.9%)	0.89
Fever	152 (32.3%)	191 (33.5%)	0.67
Gastrointestinal adverse event	66 (14.0%)	144 (25.3%)	**<0.001**
Any AE related healthcare attendance	48 (10.2%)	51 (8.9%)	0.47

Statistical significance (p-value <0.05) is highlighted in bold. Abbreviations: HRV = human rotavirus vaccine, N = number in group, NIP = national immunization program.

Table S3. Baseline characteristics and solicited AE for receipt of primary series with or without HRV per infant, for off-label subgroup of high-risk premature infants (gestational age < 27 weeks)

Characteristic	NIP vaccinated (N=5)	NIP + HRV vaccinated (N=22)
Gestational age in weeks+days (mean, SD)	26+1 (0+4)	26+0 (0+5)
Birthweight in grams (median, IQR)	925 (805-1045)	820 (523-1199)
Small for gestational age (yes,%)[£]	0	3 (13.6)
Sex (male, %)	2 (40.0)	12 (54.5)
Multiple birth (yes, %)	0	6 (27.3)
Congenital pathology (yes, %)	0	0
Age at first vaccination in days (median, IQR)	63 (54-72)	72 (45-99)
Concomitant NIP+HRV administration	NA	10 (45.5)
Sibling (yes, %)	1 (20.0)	4 (18.2)

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Characteristic	NIP vaccinated (N=5)	NIP + HRV vaccinated (N=22)
Parental education#		
High	3 (60.0)	15 (62.8)
Medium	2 (40.0)	6 (27.3)
Lower	0	1 (4.5)
Parental background ^		
European	3 (60.0)	14 (63.6)
Non-European		4 (18.2)
Mixed	2 (40.0)	4 (18.2)
Average parental age in years (median, IQR)	30.5 (17.7-43.3)	34.5 (27.5-41.5)
Parent reported solicited adverse event		
Any solicited adverse event	4 (80%)	15 (68%)
Fever	3 (60%)	8 (36%)
Gastrointestinal adverse event	2 (40%)	4 (18%)
Any AE related healthcare attendance	3 (60%)	8 (36%)

For the subgroup of infants with GA before 27 weeks, we only took those receiving care in a hospital with a policy to vaccinate off-label, as our study population. Percentages are derived excluding subjects with missing data on the variable. £ Based on 10th percentile perinatal growth curves. # Based on highest parental educational level. ^Based on parental background and categorized according to world population by country. Abbreviations: N = number in group, SD = standard deviation, IQR = interquartile range, NIP = national immunization program, HRV = human rotavirus vaccine.

Table S4. Solicited AE following receipt of NIP vaccines or concomitant NIP+HRV vaccination as part of the primary series in off-label subgroup of high-risk premature infants (gestational age < 27 weeks)

Reported adverse events after primary series vaccine administrations	Any NIP vaccination (N=14)	Concomitant NIP+HRV vaccination (N=17)
At least one solicited adverse event	9 (64.3%)	7 (41.2%)
Fever	4 (28.6%)	4 (23.5%)
Gastrointestinal adverse event	2 (14.3%)	2 (11.8%)
Any healthcare attended	3 (21.4%)	3 (17.6%)
Hospitalization	3 (21.4%)	2 (11.8%)

For the subgroup of infants with GA before 27 weeks, we only took those receiving care in a hospital with a policy to vaccinate off-label, as our study population. #1 missing information on solicited adverse events. Abbreviation: NIP = national immunization program, HRV = human rotavirus vaccine, N = number in group

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Exploration of potential bias induced by complete case analyses

Comparing infants for whom complete data were available on vaccination status to those with missing information, we only observed differences in variables that were not associated with occurrence of solicited AEs in univariate analysis (proportion of infants from multiple birth, median parental age and family educational level, **table S5**); we therefore conclude missing information is independent of the outcome of solicited AE and complete case analysis induced little bias in our study results.

Table S5. Infants with missing data versus complete cases

Characteristic	Missing (N=59)	Complete (N=960)	p-value
Gestational age in weeks+days (mean, SD)	32+1 (2+3)	32+2 (2+4)	0.59
Birthweight in grams (median, IQR)	1640 (825-2465)	1780 (930-2630)	0.42
Small for gestational age (yes,%)[£]	25 (42.2)	288 (30.0)	0.05
Sex (male, %)	28 (47.5)	532 (55.4)	0.23
Multiple birth (yes, %)	36 (61.0)	714 (74.4)	**0.02**
Congenital pathology (yes, %)	3 (5.1)	82 (8.5)	0.35
Age at first vaccination in days (median, IQR)	60 (41-79)	61 (45-77)	0.77
Sibling (yes, %)	6 (13.6)	210 (21.9)	0.20
Parental education[#]			0.03
High	30 (62.5)	728 (75.8)	
Medium	14 (29.2)	206 (21.5)	
Lower	4 (8.3)	26 (2.7)	
Parental background[^]			0.57
European	42 (84.0)	823 (85.7)	
Non-European	4 (8.0)	46 (4.8)	
Mixed	4 (8.0)	91 (9.5)	
Average parental age in years (median, IQR)	30.5 (25.0-36.0)	33.0 (27.5-38.5)	**0.03**

Percentages are derived excluding subjects with missing data on the variable. Statistical significance (p-value <0.05) is highlighted in bold. [£] Based on 10th percentile perinatal growth curves. [#] Based on highest parental educational level. [^]Based on parental background and categorized according to world population by country. Abbreviations: HRV = human rotavirus vaccine, N = number in group, SD = standard deviation, IQR = interquartile range.

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Appendix B

Term infants with congenital disorders

A total of 763 infants were born with a GA >37 weeks and with at least one congenital disorder, flowchart **figure S2**. Among the 118 HRV vaccinated infants, two vaccine related SAE were reported. Both concerned hospitalization 12 and 19 days after first dose of HRV, respectively, no information on second HRV dose was available. The first case was classified as (potentially) vaccine induced. The second as vaccine failure, sequencing was performed revealing a G3P8 genotype. Among 134 infants participating in the before-after cohort study, 79 infants were immunized with the NIP primary series vaccines and 55 infants with NIP and HRV vaccines, characteristics are shown in **table S6**. Out of all vaccine moments, 49.1% (79/161) experienced a solicited AE versus 59.1% (13/22), respectively (**table S7**).

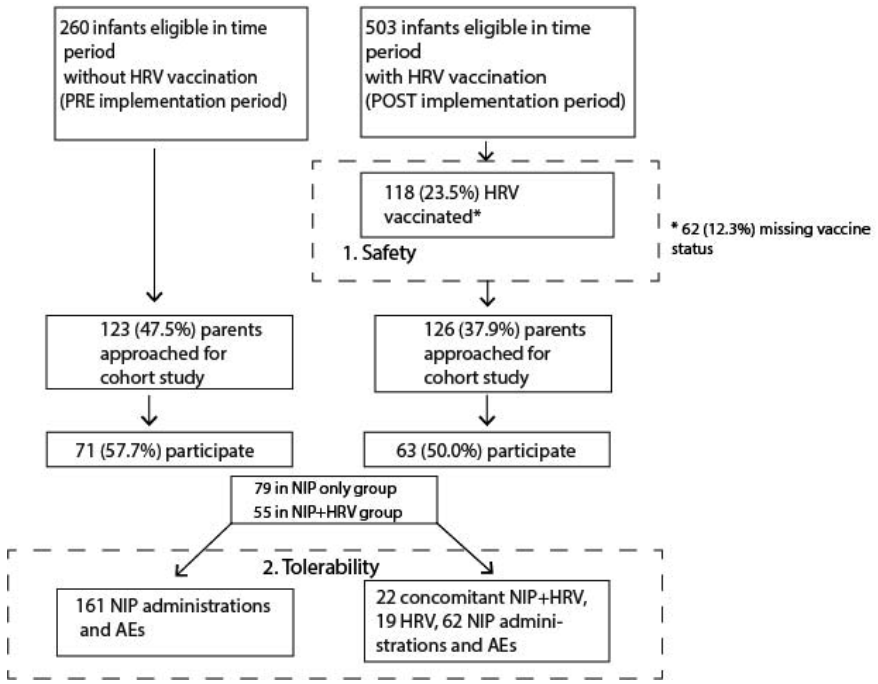

Figure S2. Flowchart of term infants with congenital disorders and cohort study-participants

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Table S6. Characteristics of term infants with congenital disorders and solicited AE for receipt of the primary series with or without HRV per infant

Characteristic	NIP vaccinated (N=79)	NIP+HRV vaccinated (N=55)	p-value
Mean gestational age in week + days (SD)	39+2 (1+2)	39+0 (1+3)	0.22
Median birthweight in grams (IQR)	3340 (2685-3995)	3330 (2525-4135)	1.0
Sex (male, %)	44 (55.7)	32 (58.2)	0.76
Number of congenital disorder, n (%)			0.25
1	55 (69.6)	7 (67.3)	
2	21 (26.6)	11 (20.0)	
> 2	3 (3.8)	7 (12.7)	
Type of congenital disorder, n (%)			
Neurodevelopmental	4 (5.1)	2 (3.6)	1.0
Cardiovascular	31 (3.2)	28 (50.9)	0.18
Pulmonary	2 (2.5)	5 (9.1)	0.12
Chromosomal	14 (17.7)	90 (18.2)	0.95
Perinatal	1 (1.3)	1 (1.8)	1.0
Other*	36 (45.6)	23 (41.8)	0.67
Age at first vaccination (median, IQR)	62 (40-84)	61 (34-88)	0.86
Concomitant NIP+HRV administration	NA	10	NA
Sibling (yes, %)	34 (49.3)	18 (34.6)	0.11
Parental education#			0.13
High	46 (65.7)	9 (75·0)	
Medium	16 (22.9)	12 (23.1)	
Lower	8 (11.4)	1 (1.9)	
Parental background^			0.90
European	60 (85.7)	45 (86.5)	
Non-European	10 (14.3)	7 (13.5)	
Average parental age in years (median, IQR)	33.5 (27.5-39.5)	34·3 (27.7-40.9)	0.49

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Characteristic	NIP vaccinated (N=79)	NIP+HRV vaccinated (N=55)	p-value
Parent reported solicited AE			
Any solicited AE	57 (72.2)	46 (83.6)	0.12
Fever	41 (51.9)	34 (61.8)	0.26
Gastrointestinal AE	20 (30.8)	22 (41.5)	0.23
Any AE related healthcare attendance	8 (10.1)	4 (7.2)	0.40

Percentages are derived excluding subjects with missing data on the variable. Statistical significance (p-value <0.05) is highlighted in bold. *Infants can have multiple congenital disorders, in category Other there are duplicates. #Based on highest parental educational level. ^Based on parental background and categorized according to world population by country. Abbreviations: N = number in group, IQR = interquartile range, SD = standard deviation.

Table S7. Solicited AE following receipt of NIP vaccines or concomitant NIP+HRV vaccination as part of the primary series in term infants with congenital disorders

Reported AE after primary series vaccine administrations	Any NIP vaccination (N=161)	Concomitant NIP+HRV vaccination (N=22)
At least one solicited AE	79 (49.1%)	13 (59.1%)
Fever	47 (29.2%)	4 (18.2%)
Gastrointestinal AE	11 (6.8%)	5 (22.7%)
Any healthcare attended for AE	8 (5.0%)	1 (4.5%)
Hospitalization	3 (1.9%)	0

Abbreviations: NIP = national immunization program, HRV = human rotavirus vaccine, N = number in group

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Chapter 6

Non-specific effects of human rotavirus vaccination in medical risk infants in the Netherlands

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Abstract

Background: The WHO recommends research into non-specific effects of vaccination. For rotavirus vaccines, these have not yet been well established. We studied non-specific effects using data from a quasi-experimental before-after study comparing cohorts of rotavirus vaccinated and unvaccinated infants with medical risk conditions up to 18 months of age.

Methods: Infants were enrolled at six weeks of age before and after a stepped-wedge implementation of a risk-group based rotavirus vaccination program. Other infant vaccinations were administered according to the Dutch National Immunization Program and similar in both periods. Non-specific effect outcomes were prospectively collected using monthly questionnaires and included acute hospitalization (excluding for acute gastro-enteritis), monthly incidence of acute respiratory illness and eczema. We used time-to-event analysis and negative binomial regression to assess the effect of at least one dose of rotavirus vaccination for each of these outcomes.

Findings: The analysis included 496 rotavirus unvaccinated and 719 vaccinated high-risk infants. In total, 1067 (88%) were premature, 373 (31%) small for gestational age and 201 (17%) had a congenital pathology. The adjusted hazard ratio for first acute hospitalization was 0.91 (95%CI 0.76;1.16) for rotavirus vaccinated versus unvaccinated infants. Adjusted incidence rate ratio for acute respiratory illness was 1.05 (95%CI 0.96;1.15) and for eczema 0.89 (95%CI 0.69;1.15).

Interpretation: The results suggest no, or minimal non-specific effects from rotavirus vaccination on non-target diseases in high-risk infants.

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Introduction

Beneficial non-specific effects have been attributed to (live attenuated) vaccines in both adults and children.[1] Non-specific effects are defined as effects of vaccines on morbidity and mortality that are not explained by the prevention of the target diseases,[2] or as resistance towards unrelated pathogens in vaccine recipients.[3] For instance, since the introduction of the first human vaccine, Vaccinia, reductions in measles and scarlet fever were observed besides the effect on smallpox.[3] The proposed mechanism for non-specific effects on non-target infections and hospitalizations is trained immunity.[1] Trained immunity is induced by epigenetic reprogramming that results in enhanced innate immune responses to reinfection and to non-target pathogens.4 Immunological studies in infants and adults established that vaccines can induce activation of innate immune cells against other than target diseases.[5–8] The duration of these beneficial non-specific effects is thought to last between three months and one year or until the next (non-live) vaccine is given.[1,9]

Epidemiologic studies suggest that bacillus Calmette-Guérin (BCG) vaccination can offer protection against respiratory infections and (recurrent) bladder cancer.[10] A recent randomized controlled trial showed that neonatal BCG vaccination prevented non-tuberculosis infectious diseases in the first six weeks of life.[11] For the Measles Mumps Rubella (MMR) vaccine, a reduction in childhood mortality was observed mediated by prevention of respiratory infections.[12] For Oral Polio vaccine (OPV) research indicated lower hospitalization rates and protection against otitis media.1 However, conflicting reports on reduction in all-cause case fatality after OPV have been published.[13–15]

Another observation from epidemiological studies is a non-specific effect on atopy. There are two reports of reduced atopy after BCG vaccination,[16,17] however a randomized controlled trial found no effect but a potential trend towards less (severe) eczema.[18] The mechanism resulting in atopy prevention is less well studied, the Th1 stimulating property of live-vaccines may prevent allergic sensitization and reduce atopy (which is Th2 mediated).[17,19]

Yet, two systematic reviews on routine childhood vaccinations and non-specific effects concluded that low quality studies and heterogeneity of the available evidence should raise caution in interpretation.[20,21] The World Health Organization recommends more research towards non-specific effects of vaccines.[22,23]

Rotavirus vaccines are live-attenuated and orally administered, like OPV. It is hypothesized that rotavirus vaccination can therefore induce beneficial non-specific effects through similar mechanisms, but this has been little studied thus far. One study reported a decrease of 31% in non-rotavirus hospitalization rates in the 60 days following rotavirus vaccination, but misclassification of rotavirus infections could not be completely ruled out.[24] To our knowledge, no literature is available on non-specific effects of rotavirus vaccination on respiratory infections or atopy.

We explored potential non-specific effects in a prospective cohort of medical risk infants in the

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Netherlands who did or did not receive rotavirus vaccination in a quasi-experimental setting.

Methods

For a detailed description of the study we refer to a previous publication (**chapter 4**). In short, thirteen Dutch hospitals with a neonatal intensive or high care ward participated in the project that combined the implementation of rotavirus vaccination program for medical risk infants with a prospective before-after cohort study. During both periods with and without rotavirus vaccination, infants were recruited at six weeks of age if they had at least one medical risk condition; 1) prematurity (gestational age < 36 weeks), 2) low birthweight (< 2500 grams) and/or 3) congenital pathology (list in **chapter 4**), and received prolonged pediatric care between six and 14 weeks of postnatal age at the participating site. Hospitals entered the study in a stepped-wedge approach and each site implemented rotavirus vaccination in routine care for medical risk infants between months 12-18 of the project. The schedule for other routine childhood immunizations according to the National Immunization Program (NIP) was left unchanged (**Figure 1**). Post-implementation, the human rotavirus vaccine (HRV, Rotarix, GSK, Belgium) was used at all sites. HRV was given in a two-dose schedule, with first dose preferably between six and nine weeks of age and the second dose with a minimum interval of four weeks and no later than 24 weeks of age. The first dose was administered by a physician in participating hospitals, the second dose was given by parents at home after detailed instruction.

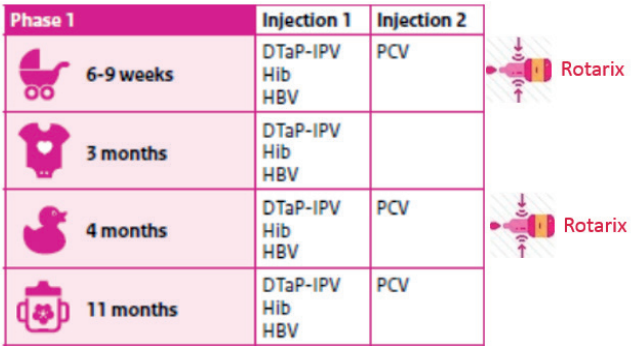

Figure 1. Vaccination schedule RIVM@2021.

Legend: Rotarix = human rotavirus vaccination

Abbreviations: DTaP-IPV = Diphteria Tetanus accelular Pertussis-Inactivated Polio vaccine, Hib = Haemophilus influenza type B, HBV = Hepatitis B vaccine, and PCV = Pneumococcal conjugate vaccine.

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Data collection

Data collection for cohort participants included monthly parental questionnaires until 18 months of age. Parents answered yes/no questions concerning acute respiratory illness (ARI) symptoms, presence of eczema symptoms and hospital admission in the past month. ARI symptoms included fever with or without nasal congestion/runny nose, cough and earache. In addition the number of days with ARI symptoms in each month was recorded. For hospitalization, the reason and number of hospital days was also collected.

Non-specific effect outcomes

We defined non-specific effect outcomes of rotavirus vaccination as the relative change in incidence of parent-reported acute hospitalization, ARI or eczema between rotavirus vaccinated and unvaccinated infants.

Acute hospitalization was defined as any hospital admission following initial post-natal discharge, excluding hospital admissions for acute gastroenteritis. Hospital admissions for scheduled medical or surgical interventions were also excluded.

Analyses

Descriptive statistics were used to compare patient characteristics and outcomes between vaccinated and unvaccinated infants. We calculated the cumulative incidence and incidence rate for each non-specific effect outcome by group status. As secondary outcomes, we calculated the cumulative acute hospitalization days and days with ARI symptoms for rotavirus vaccinated and unvaccinated infants.

Next, we used Cox regression to model the effect of at least one dose of rotavirus vaccination on time to first hospital admission, with age (between two and 18 months) as time axis. Infants were censored when lost to follow up, dropped-out, deceased or hospitalized, whichever came first. The proportional hazard assumption was visualized graphically and tested with Schoenfelds residuals. The final model with covariates was derived using log-likelihood ratio test, hazard ratios (HR) and their 95% confidence intervals (CI) were provided. Infants were categorized as rotavirus vaccinated from 28 days post-dose one onwards.

For ARI, we used a negative binomial model with offset for persontime of observation to compare the number of months with ARI between rotavirus vaccinated and unvaccinated groups. Incidence rate ratios (IRR) were obtained with their 95% CIs. We used Akaike Information Criterion to select the final model. The analysis was repeated for the outcome months with eczema.

For the secondary outcome of cumulative days hospitalized or days with ARI complaints we used a Poisson model. Because of overdispersion for hospitalization days we used a negative binomial model.

In sensitivity analyses, we separately analysed effects up to six months of age, covering the

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initial three months post-rotavirus vaccination, and up to eleven months of age, covering the period up to the booster vaccinations of the NIP. We also analysed hospitalizations for infectious diseases separately, using Cox modelling as described above.

For all models, we considered the following covariates: sex, type of medical risk condition, gestational age, breastfeeding, daycare attendance, type of hospital care (i.e. academic versus general), family educational level, parental origin, NIP vaccination status, presence of siblings in the household and season with high rates of respiratory infections (from October until April).

All analyses were performed on according to protocol cohorts, where all high-risk infants whose parents indicated willingness to vaccinate their child against rotavirus in the pre-implementation cohort are compared to infants that received at least one HRV dose in the post-implementation cohort. This information was based on a parental questionnaire at start of the study, see **chapter 4**.

We performed complete case analyses, missing information is documented. As statistical software SPSS version 25 and RStudio version 5.0 were used, with packages *MASS, fmsb, survival* and *survminer*.

Results

A total of 1482 high-risk infants with one or more medical risk conditions were enrolled in the study. The population for analysis included 719 rotavirus vaccinated infants and 496 infants whose parents indicated willing to vaccinate against rotavirus, but who were enrolled before rotavirus vaccination was implemented. In total, 1067 infants (87.8%) were born premature, 373 infants (30.7%) were small for gestational age and 201 infants (16.5%) had at least one congenital condition. Baseline characteristics were comparable between the rotavirus vaccinated and unvaccinated group (**table 1**), with the exception of follow up, which was complete for 450 vaccinated infants (62.6%) and 380 (76.6%) of unvaccinated infants. The proportion of observation months during seasons with or without high rates of respiratory infections was not significantly different between the groups, ARIs occurring in-season was also not different.

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Table 1 Baseline table with characteristics of vaccinated and unvaccinated infants

	According to protocol groups protocol (ATP)		
	Pre-implementation: willing to vaccinate	Post-implementation: vaccinated	p-value
	N=496	N=719	
Male sex	286 (57.7%)	381 (53.0%)	0.11
Multiple births	115 (23.8%)	193 (26.8%)	0.15
Premature birth (gestation < 37 weeks)	432 (87.8%)	635 (88.3%)	0.79
Small for gestational age[£]	135 (27.2%)	238 (33.1%)	**0.03**
Presence of severe congenital disorder[*]	95 (19.2%)	106 (14.7%)	**0.04**
Vaccinated according to NIP	484 (97.6%)	652 (90.7%)	**<0.001**
Daycare attendance	289 (58.6%)	398 (59.6%)	0.74
Socioeconomic status[^]			0.36
Higher	352 (71.5%)	492 (74.1%)	
Moderate	119 (24.2%)	153 (23.0%)	
Lower	21 (4.3%)	19 (2.9%)	
Parental origin[#]			0.75
European parent(s)	417 (84.8%)	557 (83.4%)	
Non-European parent(s)	28 (5.7%)	38 (5.7%)	
Mixed	47 (9.6%)	73 (10.9%)	
Mean number of months with completed follow-up (SD)	16.4 (5.5)	14.9 (6.5)	**<0.001**
Number with complete follow-up until 18 months of age	380 (76.6%)	450 (62.6%)	**<0.001**
Mean proportion of months during ARI season (SD)[$]	0.50 (0.18)	0.50 (0.18)	0.75

Percentages are computed excluding subjects with missing data on the variable. Statistical significance (p-value <0.05) is highlighted in bold. [£] Based on the 10th percentile perinatal growth curves. [*]For a list of qualifying congenital disorders, see chapter 4. [^]Based on highest parental education level. [#]Based on parental origin by World population by country[25] [$] ARI season in the Netherlands is defined from October until April.[26] Abbreviations: GA= gestational age, SD= standard deviation, IQR = interquartile range, yrs= years, NIP = National Immunization Program and, N = number in group.

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Non-specific effect outcomes by vaccination status are described in **table 2**. Twenty-one hospitalizations for acute gastroenteritis were excluded from the acute hospitalizations (eleven among unvaccinated and ten among vaccinated infants, respectively). Thirty-nine percent of infants were hospitalized at least once or reported one month with eczema. For 82% of infants at least one ARI episode was reported. Incidence of any of the non-specific effect outcomes or the mean cumulative number of days hospitalized or with ARI symptoms show no significant differences (**table 2**).

Table 2 Non-specific effect outcomes for vaccinated and unvaccinated infants

	Pre-implementation: willing to vaccinate N=496	Post-implementation: vaccinated N=719
At least one acute hospitalization	212 (43.0%)	262 (39.2%)
Cumulative number of hospitalizations	367	433
Incidence of hospitalization* (95%CI)	0.54 (0.48;0.59)	0.48 (0.43;0.52)
Mean cumulative number of hospitalization days	8.3	8.2
At least one ARI	411 (83.4%)	584 (87.3%)
Cumulative number of months with ARI	1888	2660
Incidence of months with ARI (95%CI)	2.77 (2.64;2.89)	2.92 (2.81;3.04)
Mean cumulative number of days with ARI	23.2	24.5
At least one month with eczema	204 (41.4%)	270 (40.4%)
Cumulative number of reported eczema	995	1215
Incidence of months with eczema (95%CI)	1.46 (1.37;1.55)	1.33 (1.26;1.41)

Percentages are derived excluding cases with missing information (n=53). *Incidence per person year of observation. Abbreviations: N=number in group, CI = confidence interval and, ARI = acute respiratory illness.

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Based on the adjusted Cox model at least one dose of HRV was not significantly protective against first acute hospitalization (HR for time to first acute hospitalization 0.91; 95%CI 0.71;1.18, **table 3**). The final model included gestational age, presence of a congenital disorder, type of hospital care and seasonality as covariates.

In addition, no effect was observed on the occurrence of ARI (adjusted incidence rate ratio (IRR) 1.05; 95%CI 0.96;1.15), or eczema (adjusted IRR: 0.89; 95%CI 0.69;1.15, **table 3**). Comparing the secondary outcomes of cumulative or hospitalization days showed no statistically significant difference. The cumulative number of days with ARI was increased for vaccinated infants (**table 3**).

Table 3 Effect estimates of non-specific effects

	Univariate	95% CI	Multivariate estimate	Lower 95% CI	Upper 95% CI
HR for acute hospitalization*	0.85	0.66-1.09	0.91	0.71	1.18
IRR for ARI#	1.07	0.99-1.17	1.05	0.96	1.15
IRR for eczema#	0.94	0.74-1.18	0.89	0.69	1.15
Secondary					
RR Cumulative number of days hospitalized	0.91	0.66-1.25	0.83	0.59	1.16
RR Cumulative number of days with ARI	1.06	1.04-1.09	1.06	1.03	1.09
Sensitivity					
HR for infectious diseases hospitalization*	0.93	0.56-1.60	0.96	0.58	1.62
HR for acute hospitalization until 6 months*	1.08	0.76-1.53	1.20	0.85	1.71
HR for acute hospitalization until 11 months*	0.87	0.65-1.16	0.95	0.71	1.27
IRR for ARI until 6 months#	1.05	0.89-1.24	0.94	0.78	1.14

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	Univariate	95% CI	Multivariate estimate	Lower 95% CI	Upper 95% CI
IRR for ARI until 11 months[#]	1.10	0.99-1.22	1.03	0.92	1.15
IRR for eczema until 6 months[#]	0.99	0.71-1.41	0.91	0.62	1.33
IRR for eczema until 11 months[#]	0.96	0.73-1.24	0.89	0.66	1.19

[*] Adjusted for: gestational age, presence of congenital disorder, type of hospital care and seasonality. [#]Adjusted for: gestational age, daycare attendance, parental educational level, presence of sibling in the household, vaccinated according to NIP program and seasonality. Abbreviations: HR = hazard ratio, IRR = incidence rate ratio, RR = relative risk, CI = confidence interval

In sensitivity analyses we observed that the non-specific effects estimates were closer to one and non-significant when restricting to the six or eleven months of age for all non-specific effect outcomes, **table 3**. Restricting to acute hospitalizations for infectious diseases resulted in an adjusted HR of 0.96 (95%CI 0.58;1.62).

Discussion

This quasi-experimental prospective study assessed the potential non-specific effects of rotavirus vaccination among 1215 infants with medical risk conditions. Our results suggest that HRV does not offer significant protection against non-target diseases leading to acute hospitalization, or result in reduced incidence of ARI or atopy up to 18 months of age.

Non-specific effects of (live-attenuated) vaccines are increasingly being studied. Currently, 22 trials are being performed or completed on the potential protective effect of BCG vaccination against COVID-19 disease.[22] For rotavirus vaccines however, there has been very little research into non-specific effects despite their availability and widespread use for more than a decade. While some promising results of beneficial non-specific effects, in particular for BCG vaccine are available,[1,4,9,19] other studies report contradictory and less convincing results.[12–14,27] The results of our study add to the growing body of evidence hinting towards absence of non-specific effects, at least for rotavirus vaccines.

Opposite to our result, a reduction in acute hospitalization due to non-target diseases after rotavirus vaccination was reported in a previous study from the United States.[24] As a secondary analysis, this study compared hospitalization rates in the 60-day post-vaccination window for rotavirus vaccinated and unvaccinated infants, excluding hospitalizations coded as rotavirus gastro-enteritis. A reduction of 31% (95%CI 27-35%) was reported. However, coding for rotavirus hospitalizations is known to be incomplete.[28] By including hospitalizations for gastro-

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enteritis without the rotavirus specific code, misclassification may have occurred and could explain the observation mediated by direct, rather than indirect vaccine effects.

Most likely, a reduction in acute hospitalizations resulting from non-specific effects would be mediated by lowering infection incidence. This is however not supported by our findings that show a similar rate of ARI in vaccinated and unvaccinated infants. Furthermore, when restricting the analysis to hospitalizations for infectious diseases only, an even smaller effect was estimated. Alternatively, one could argue that non-specific effects following rotavirus vaccination reduce severity of ARI, rather than ARI incidence, thereby reducing the risk of hospitalization. However, no reduction was observed in days with ARI symptoms, which could be considered a proxy for severity. Another hypothesis mentions that inactivated childhood vaccinations abrogate the beneficial non-specific effects of earlier administered live vaccines. To investigate this, we conducted a sensitivity analysis in which we restricted the analysis to the time-period up to the next routine childhood vaccination at 11 months, but the effect estimate was unchanged. Jointly, our results do not support the existence of non-specific effects for rotavirus vaccines lowering overall hospitalization rates, infection incidence or severity.

For eczema, we did not find a significant effect of rotavirus vaccination either. Evidence on preventing atopy by vaccination so far is based on one underpowered randomized clinical trial and several heterogeneous low-quality observational studies.[29] Unfortunately, our study was also underpowered to draw any firm conclusion on the effect of rotavirus vaccination on eczema incidence. Our point estimate of 0.89 suggests some benefit may exist, but this warrants confirmation in other controlled studies.

In this study the majority of infants was born prematurely. While non-specific effects have also been investigated among low birthweight infants in multiple randomized trials,[6,30,31] premature infants have a higher risk of respiratory morbidity and hospitalization.[32] Therefore any non-specific effect on ARI incidence should become apparent in this specific study population. However, direct vaccine effectiveness in our study population was lower than previously anticipated (**chapter 4**). If the direct vaccine response in this population is reduced, this may also apply to non-specific vaccine effects. Overall, there is an additional need for research into non-specific effects of rotavirus vaccination in a healthy term infant population.

A few limitations of this study need to be addressed. First, we used complete cases only, missing information is documented and comparing participants with complete versus missing data did not reveal significant differences (data not shown). Loss to follow up occurred in approximately 30% of participants. By using time-to-event analysis and including an offset for observation time this was taken into account in our analyses and we therefore expect this has minimal effect on our results.

We were unable to assess severity of ARI, a discrimination in episodes by severity might further explain why the difference in acute hospitalizations was not reflected in occurrence of ARI. Instead, we assessed number of days with ARI complaints and observed no relevant difference

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between vaccinated and unvaccinated infants. Duration of disease could function as a proxy for severity.

In conclusion, rotavirus vaccination in medical risk infants was not associated with beneficial non-specific effects on (acute hospitalization due to) non-target diseases. The study results suggest that beneficial non-specific effects, as observed for some other live-attenuated vaccines, may not apply to oral rotavirus vaccines, but studies in healthy term infants are needed to further establish this.

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Supplementary Material

Table S1. Reasons for acute hospitalization, excluding acute gastroenteritis, planned treatment, surgery or examination.

Reason for hospitalization	Hospitalizations in pre-implementation: willing to vaccinate (N=367)	Hospitalizations in post-implementation: vaccinated (N=433)
Infectious (n,%)	45 (12.3)	56 (12.9)
IR per person year	0.06	0.06
Growth problems (n,%)	20 (5.4)	9 (2.1)
IR per person year	0.03	0.01
Feeding problems (n,%)	27 (7.4)	36 (8.3)
IR per person year	0.04	0.04
Non-infectious intestinal (n,%)	13 (3.5)	12 (2.8)
IR per person year	0.02	0.01
Breathing difficulties (n,%)	124 (33.8)	119 (27.5)
IR per person year	0.18	0.13
Excessive crying (n,%)	6 (1.6)	11 (2.5)
IR per person year	0.01	0.01
Other reason (n,%)	132 (36.0)	190 (43.9)
IR per person year	0.19	0.21

Abbreviatons: IR = incidence rate, n = number with characteristic, N = number in group.

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Chapter 7

Evaluation of implementing a rotavirus vaccine program targeting high-risk infants in The Netherlands

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Abstract

Background: Prior to introduction of a rotavirus vaccination program in the Netherlands, a pilot was conducted in 13 hospitals to trial the implementation process, assess feasibility and vaccine coverage. A targeted rotavirus vaccination program for high-risk infants was scheduled to be introduced in 2020.

Methods: Between May 2016 and October 2017 pediatric and neonatal departments in 13 hospitals implemented targeted rotavirus vaccination for medical risk infants, including those with prematurity, low birth weight and/or a congenital disorder. Rotavirus vaccination was available as standard care without charge. Vaccination status of all eligible infants was documented and feasibility was evaluated based on surveys and in-depth interviews.

Results: Overall mean vaccine coverage among eligible medical risk infants was 52.3% (95%CI 50.8;53.7%) and ranged between 24.4% (95%CI 21.3;27.5%) and 83.4% (95%CI 79.9;87.0%) per site and between 22.9% (95%CI 18.7;27.1%) and 58.3% (95%CI 56.3;60.4%) per type of medical risk condition. Only 34.1-38.5% of surveyed parents were informed of the vaccine as part of standard care. In-depth interviews revealed infant, healthcare and vaccine related barriers and facilitators of this implementation program.

Conclusion: Implementation of a hospital-based targeted rotavirus vaccination program, resulted in suboptimal vaccine coverage among high-risk infants and was not introduced in 2020. Involvement of the national immunization program existing structure would be preferable in order to reach a substantial larger proportion of the risk-groups.

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Background

Immunization in childhood has been found a highly (cost-)effective preventive measure[1]. However successful introduction of new vaccines is dependent upon multiple factors[2]. Requirements for successful implementation of new vaccines are structured organization of the immunization program, raising awareness and providing adequate information to parents, favorable vaccine characteristics, and a feasible implementation strategy. Specifically for childhood immunization, parental conceptions influence the willingness to receive vaccination and therefore vaccination coverage[3–5]. In addition, the attitude and perceptions of health care providers (HCP) towards vaccination have been identified as important determinants for implementation success[6–8]. Vaccination coverage is a key performance indicator to evaluate vaccine strategy and implementation policy.

As of 2020, at least 98 countries have implemented rotavirus vaccination in their National Immunization Program (NIP)[9]. Globally licensed rotavirus vaccines are Rotarix (GSK, Belgium) and RotaTeq (Merck and Co, USA). They both have a high efficacy against severe rotavirus gastroenteritis ranging from 80 to 100% in high-income countries[10–14]. The two vaccines are generally well-tolerated. In the Netherlands, the health council advised, in 2007, against universal rotavirus vaccination, due to insufficient evidence on a specific risk group for vaccination and rotavirus genotype distribution[15]. Because it was demonstrated that infants with medical risk conditions (MRC) had increased disease burden[16], a targeted vaccination strategy was piloted for infants with MRC only. This Risk-group Infant Vaccination Against Rotavirus (RIVAR) project started in 2014. For program execution, pediatric secondary and tertiary care facilities were made responsible, instead of well-baby clinics that offer all routine childhood vaccines of the NIP. The main objectives of the RIVAR project were: 1) to determine vaccine effectiveness of rotavirus vaccination among infants with MRC and 2) to assess the feasibility of implementing a targeted rotavirus vaccine program in secondary and tertiary hospitals in the Netherlands. The second aim of the RIVAR project is described in this implementation study.

Methods

RIVAR project and intervention

A full description of the project is published at www.trialregister.nl (NTR5361)[17] and vaccine effectiveness is described elsewhere (**chapter 4**). In brief, rotavirus vaccination was implemented in thirteen Dutch hospitals with a Neonatal Intensive Care Unit (NICU) or post-Intensive Care (IC) ward. This intervention was added to standard care for premature infants (gestational age (GA) below 36 weeks), infants with a low birthweight (LBW) less than 2500 grams and/or infants with severe congenital pathology (for a list of conditions, see **chapter 4**). In order to be eligible for rotavirus vaccination, infants should receive in- or out-patient care in the participating hospitals between six and fourteen weeks of age, corresponding with the time window for the first dose of rotavirus vaccine administration. Rotarix, the human rotavirus vaccine (HRV) was

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used within the RIVAR project. Exclusion criteria were: known hypersensitivity to the vaccine, severe diarrhea, previous intussusception or severe immunodeficiency. All hospitalized infants in a participating hospital were screened and basic patient characteristics were entered into a digital Case Report Form (CRF). At the age of five months, the HRV immunization status was retrieved from medical records for all eligible infants and recorded in the CRF.

A subset of eligible infants participated in the before-after cohort-study and was followed until 18 months of age for the occurrence of acute gastroenteritis.

Implementation of a targeted rotavirus vaccination program

Hospitals implemented the rotavirus vaccination program at different time points and entered the project one year prior to implementation of HRV (step wedged design). During a preparatory pre-implementation year the organizational and logistical infrastructure for patient selection and vaccine administration was set up in each hospital. Various educational activities targeting physicians and nurses were organized, such as group-presentations, e-learnings, and workshops. Nurses received practical tutorials for administration. We distributed pocket cards among medical doctors with eligibility criteria, (contra-) indications, the vaccination schedule and possible side effects of HRV. Posters and hand-outs were distributed to clinical wards and out-patient clinics highlighting key elements of the program. Shortly before implementation, email newsletters were distributed to all HCP involved in the upcoming HRV implementation. The implementation of HRV was supported by research personnel and -nurses. Administration of HRV first dose was scheduled during hospital stay or combined with a planned out-patient visit. The second dose of HRV was administered by parents at home, after they received instructions. Cold chain was guaranteed by a cooling device upon collection from the hospital. According to the product information, HRV can be administered in premature infants of at least 27 weeks gestation. However, national guidelines vary in their advice for HRV in these infants. Decisions about allowing administration of HRV on the in-patient-ward and to infants born before a gestational age of 27 weeks were left to the discretion of local policy in participating hospitals. Regular audits were performed to guide the implementation throughout the project. Every two months the interim vaccination coverage was communicated to all hospitals. The program execution was discussed in order to identify improvements and caveats.

Evaluation survey

Among a sample of HCP and parents involved with rotavirus vaccination in the participating hospitals we performed a survey to evaluate the implementation of the HRV program. The survey focused on study-specific and program specific themes. The themes were divided into: 1) rotavirus vaccination information provision, 2) program execution and 3) preference of rotavirus vaccination strategy. Information provision and execution were assessed using

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statements that could be scored on a five-point Likert-scale (from completely disagree to fully agree). Statements like "The information provided to me about rotavirus vaccination was easy to understand" were proposed for information provision and "I received timely information; I had sufficient time to decide whether I wanted to vaccinate my child" for the execution. The survey contained multiple choice questions on preference of rotavirus vaccination strategy, for instance preferred setting for patient selection, indication and vaccine delivery. The survey questionnaires are attached as supplementary material. A sample of five to ten HCP and parents per participating hospital were invited to complete the questionnaires. Both parents of vaccinated and non-vaccinated infants were invited to participate.

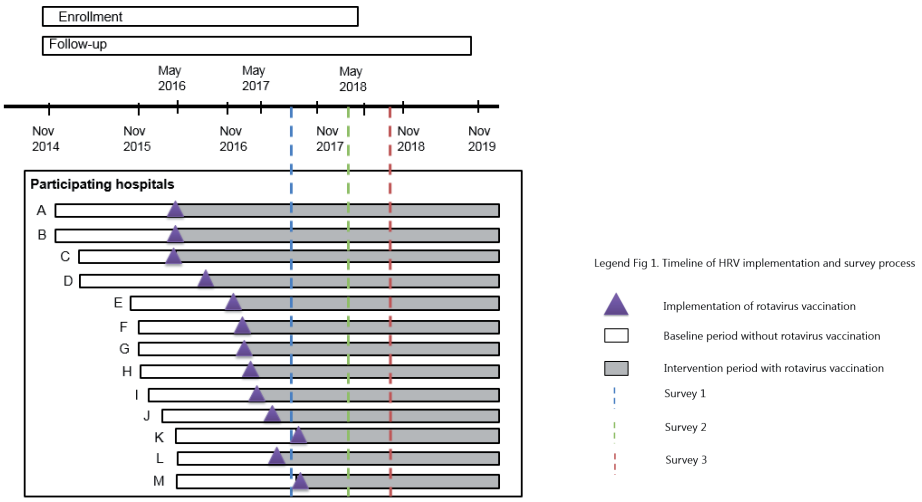

Figure 1. Timeline of HRV implementation and survey process

Invitations were sent via newsletters, RIVAR Facebook page and personal email. Questionnaires were developed and distributed via SurveyMonkey©. There were three surveys at different time points (**Figure 1**). Hospitals participated in the different surveys at least three months after implementation of HRV.

In addition, the first two themes (1. information provision and 2. program execution) were further explored by the in-depth interviews. We conducted semi-structured interviews with HCP and research personnel on barriers and facilitators encountered during the implementation process. We approached different type of HCP and research personnel from a selection of participating hospitals. The interviews were performed by JvD, documented and verified with each interviewee.

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Outcome definitions

The primary feasibility outcome was the overall vaccination coverage and timeliness of vaccination among eligible infants. Vaccination coverage was calculated as the proportion of eligible infants that received at least one of the two doses of HRV. Timeliness of vaccination was defined as administration of the first dose of HRV between six and fourteen weeks of postnatal age, preferably between six and nine weeks of age. Timeliness for administration of the second dose was determined as an interval of at least four weeks post-dose one and no later than 24 weeks of postnatal age. We assessed differences in vaccination coverage and timeliness between infants with different types of MRC and between participating hospitals (i.e. academic versus general hospital). In addition, vaccination coverage relative to time since implementation is reported. Because only five hospitals had a policy of vaccinating infants with GA < 27 weeks, we reported that group separately (see supplement).

From the survey results, we defined several indicators of implementation success. These included: 1) the proportion of HCP and parents that agreed with clear information provision, 2) the proportion of HCP and parents that agreed with timely and effective execution of the program and 3) the vaccination strategy chosen by the highest proportion of HCP and parents as best approach. Any differences between type of HCP (i.e. medical doctor versus non-medical doctor) and parents of vaccinated or unvaccinated infants was also presented. In this paper we focused on program specific themes as described above, study-specific outcomes are described in the supplementary material. Theme related quantitative survey results were integrated with qualitative interview outcomes.

Analyses

We used Pearsons Chi-square, Fisher Exact, Students'T or non-parametric tests for comparison of vaccinated and unvaccinated infants, depending on the measured variable and its distribution.

Survey statements scored on the five-point Likert-score were dichotomized in agree and neutral/disagree.

Theoretical thematic analysis of semi-structured interviews was done by JvD and PB. The barriers, facilitators and themes were identified and discussed in group meetings 18. After consensus was achieved, within each theme the different barriers and facilitators were distinguished.

SPSS version 25.0 and RStudio version 5.0 were used as statistical software.

This paper used the StaRI recommendations for reporting implementation studies, and the COREQ checklist for qualitative research[19,20].

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Results

Evaluation of the intervention

Among 4958 screened infants in 13 hospitals post-implementation of the rotavirus vaccination program, 4621 had a GA of ≥27 weeks and were eligible for HRV vaccination. In total, 2370 infants were vaccinated with at least one HRV dose, resulting in overall mean vaccination coverage of 52.3% (95%CI 50.8-53.7%). The differences in coverage among types of MRC and hospitals (academic versus general) are presented in **table 1**. Over time the vaccine coverage slightly improved, with a maximum between 6 and 12 months since implementation. The subset of infants participating in the cohort-study had a significant higher vaccination coverage compared to those not participating (**table 1**). Vaccination coverage per site ranged between 24.4% (95%CI 21.3;27.5%) and 83.4% (95%CI 79.9;87.0%). Timeliness of first dose HRV administration between six and nine weeks of age was 51.8% (1213/2340 infants), 2164 infants (92.5%, 95%CI 91.4;93.5%) were vaccinated within 14 weeks of age. For the second HRV dose limited information was available due to at home self-administration, 665 out of 772 infants (86.1%, 95%CI 83.7;88.6%) were vaccinated on time. There were no statistical significant differences in timeliness by type of medical risk group or type of hospital.

Table 1. Comparison of characteristics among HRV vaccinated and unvaccinated infants (GA ≥ 27 weeks) with medical risk conditions

	Vaccinated N=2370	Unvaccinated N=2162	p-value
Type of MRC			
Premature	1317 (58.3%)	941 (41.7%)	**0.00**
SGA	144 (54.8%)	119 (45.2%)	
Premature and SGA	662 (57.5%)	490 (42.5%)	
Congenital pathology	89 (22.9%)	299 (77.1%)	
Premature and congenital pathology	78 (36.4%)	136 (63.6%)	
SGA and congenital pathology	31 (28.7%)	7 (71.3%)	
Premature, SGA and congenital pathology	46 (33.1%)	93 (66.9%)	
Birthweight <2500 grams	2 (66.7%)	1 (33.3%)	
Hospital type			
Academic	438 (28.2%)	1116 (71.8%)	**0.00**
General	1932 (64.9%)	1046 (35.1%)	
Time since implementation			

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	Vaccinated N=2370	Unvaccinated N=2162	p-value
0-6 months	429 (47.7%)	470 (52.3%)	**0.001**
6-12 months	486 (56.5%)	374 (43.5%)	
> 12 months	1455 (52.5%)	1318 (47.5%)	
Participant in cohort study			
Yes	695 (87.3%)	101 (12.7%)	**0.00**
No	1675 (44.8%)	2061 (55.2%)	

Percentages are derived excluding missing information. Statistical significance (p-value <0.05) is highlighted in bold. Abbreviation: SGA = small for gestational age, MRC = medical risk condition

Evaluation of the implementation strategy

A total of 136 HCP completed the survey (**table 2**). Of these, 42 were actively approached and 94 responded on invitations for the survey in the newsletter; 123 were female (89.8%), and 51 (38.1%) were medical doctor versus 56 (40.9%) nurses. In total, 194 parents of vaccinated infants and 19 parents of unvaccinated infants completed the SurveyMonkey; 44 parent pairs were actively approached and 169 parent pairs responded to the survey invitation in the newsletters or on Facebook. For a flowchart of total approached and responded, see supplement **figure S2**. The total number of survey respondents and their characteristics are presented in **table 2.** Among parents of vaccinated infants, 168 (87.0%) were female, median age 33.0 years (IQR 5.0) and 131 had a high educational level (68.6%). Among parents of unvaccinated infants that completed the questionnaire, 17 (89.5%) were female, median age 32.0 years (IQR 6.0) and 15 (78.9%) highly educated. For the interviews, five HCP were approached and three responded.

Table 2. Characteristics of participants of evaluation survey

Characteristic	HCP (N=136)	Parents of vaccinated infants (N=194)	Parents of unvaccinated infants (N=19)
Sex (female, %)*	123 (89.8)	168 (87.0)	17 (89.5)
Age (median years, IQR)*	42.0 (20.0-62.0)	33.0 (28.0-38.0)	32.0 (26.0-38.0)
Educational level (n,%)*			
Higher/medical doctor	51 (38.1)	131 (68.6)	15 (78.9)
Medium/other HCP	83 (61.9)	48 (25.1)	4 (21.1)
Lower		12 (6.3)	

* One missing for sex, three missing for age and education among parents of HRV vaccinated infants. Abbreviations: HCP = healthcare providers, N = number in group, IQR = interquartile range, n = number with characteristic.

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Theme 1. Information provision

Among HCP, 93 (72.1%) stated that the information provision was clear and 70/115 (60.9%) felt sufficiently informed about rotavirus vaccination. Among parents of vaccinated infants, 149 (85.6%) were positive about the amount of information they received and, 159 parents (91.4%) thought the provided information was clear. Five parents of unvaccinated infants (29.4%) stated they did not receive enough information to base their decision on and three found the information was unclear (17.6%). A pediatric research nurse in a secondary care facility mentioned during the interview on information provision:

"*The age period for the first dose is actually no real limitation, when parents of eligible infants are properly informed at eight weeks of age there is ample opportunity to vaccinate them in time.*" (Int. HCP2).

The other two interviewees mentioned parents of eligible infants were overwhelmed and wondered how much of the information provided shortly after birth on this new vaccine would linger.

"*In the two years the project has run in both hospitals I have spoken to many parents of eligible children. Most parents were overwhelmed by the situation they were in." (Int. HCP1) "How much information lingers shortly after delivery?*" (Int. HCP3).

Explicitly focusing on the favorable characteristics of HRV administration in the information provision; being non-invasive (oral), quick, the possibility for concomitant administration with other NIP vaccines, was mentioned as facilitating factor.

"*… parents of young premature infants are benevolent to vaccination. Because of oral administration, they are more inclined to choose rotavirus vaccination for their child.*" (Int. HCP2).

Theme 2. Program execution

Among HCP, 96 (76.2%) agreed to the statement "In my hospital all eligible infants are routinely informed about rotavirus vaccination" and 66 HCP (52.8%) agreed that the vaccine was routinely offered to eligible infants. Among parents of vaccinated infants, 139 (80.3%) agreed to the statement "I received timely information about rotavirus vaccination; I had sufficient time to decide whether I wanted to vaccinate my child." whereas among parents of unvaccinated infants, eight (50.0%) agreed to this statement. Among parents of vaccinated infants, 59 parents (34.1%) agreed to the statement "I was first informed about rotavirus vaccination by a pediatric doctor/nurse as part of standard care", and among parents of unvaccinated infants five (38.5%) agreed.

An infant related barrier was the health status of the infant during the age-window for first rotavirus vaccination, as was expressed by an on-site research nurse:

"*In general the infants admitted to the tertiary center, especially in the case of severe congenital*

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anomaly, need more complex care, often involving a multitude of pediatric specialists. Vaccination as a rule is only appropriate when the infant is stable which is often not the case during admission in tertiary care." (Int. HCP1).

The implementation of rotavirus vaccination protocol was adapted to local policy by each participating hospital. Sometimes, additional restrictions on administration were adopted, limiting opportunities for vaccination for the most vulnerable infants. One of the interviewees remarked:

"*This center has an in-hospital vaccination policy which stipulates not to offer vaccination to infants born under 27 weeks of gestation; not to administer vaccination in the NICU setting; to offer vaccination only at time of discharge on the infant/surgical ward or during outpatient visits.*" (Int. HCP1). In addition, the variation in out-patient follow-up for these infants made it difficult to implement a vaccination program in an uniform and standardized way. As was clarified by a neonatologist:

"*There is no national follow-up program for eligible infants, in-hospital administration of first dose HRV is the best way for infants born before 32 weeks of gestation... Almost all eligible infants are born in a hospital, we do see these infants. The vast majority in our hospital is however born at term. The eligible infants represent approximately 20%, prematurity is the main group, smaller group of dysmature and infants with a congenital disorder even smaller. It is complex and individual care… Rotavirus vaccination is not part of the standard thought processes, not routine.*" (Int. HCP3).

Awareness, belief and attitude towards rotavirus vaccination as new standard of care for infants with MRC was mentioned both as a barrier and as facilitator. An on-site research nurse said:

"*The institution is proud to be a participating hospital, feels it can offer it's patients additional care and even issued a press release once the rotavirus vaccination became available… However, the quick turnover of pediatric residents – most of the time unfamiliar with the RIVAR project – does not help the implementation process.*" (Int. HCP1).

For the execution of the program hospital nurses might be the best option, suggested by a HCP: "*Nurses provide stable care for these infants. In a training hospital there is a high turnover of residents and it takes time to create awareness among new physicians... Well-baby clinic physicians can function as safety net….*" (Int. HCP3).

On the other hand, endorsement by a medical doctor can improve willingness to vaccinate: "*In the tertiary center it was often just the research nurse discussing and explaining the need for rotavirus vaccination while it was never mentioned by the pediatrician (or resident). The implicit message to parents might be that it is of inferior importance since it is not discussed as part of current practice.*" (Int. HCP1).

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Theme 3. Preference of rotavirus vaccination strategy

A majority of HCP 74/122 (60.7%) were in favor of providing HRV for infants with MRC, free of charge; 47 HCP (38.5%) thought HRV should be provided for all infants in the Netherlands, as part of the NIP. The preferred setting for targeted vaccine indication was the second and third line of care according to 64/75 (85.3%) of overall HCP, these were mainly other (non-medical doctor) HCP. Differences between medical doctors and other HCP in their experience with the HRV program are shown in **table 3**, all are statistically significant. Among parents of vaccinated infants, 94 (55.0%) were in favor of a universal vaccination program as opposed to nine parents of unvaccinated infants (69.2%). Of vaccinated infants, 72 parents (42.1%) choose for well-baby clinics as preferred setting for vaccine delivery. Differences between parents of vaccinated and unvaccinated infants in their experience with targeted rotavirus vaccination are presented in **table 4**, perceived timely information differed significantly. Reasons for refusing vaccination were; administration not possible within age limits (n=4), not being offered the vaccine (n=2), off-label (n=1), and perceived risks did not outweigh potential benefits (n=6).

Table 3. Health care providers' experience with the targeted rotavirus vaccination program

Statement	Medical doctors (N=51)	Other HCP (N=84)	p-value
Information provision about implementation of rotavirus vaccination in my hospital was clear (n agree with statement, %)	29/48 (60.4)	64/80 (80.0)	**0.02**
Rotavirus vaccination should be available for high risk children (n, %)	34/44 (77.3)	40/78 (51.3)	**0.005**
Second and third line care centers should be responsible for providing rotavirus vaccination (n, %)	10/34 (29.4)	21/42 (50.0)	**0.01**

Percentages are derived excluding respondents with missing information. Statistical significance (p-value <0.05) is highlighted in bold.

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Table 4. Parental experience with the targeted rotavirus vaccination program

Statement	Vaccinated infants (N=194)	Unvaccinated infants (N=19)	p-value
The information provided to me about rotavirus vaccination was clear (n agree with statement, %)	159/174 (91.4)	14/17 (82.4)	0.22
I was first informed about rotavirus vaccination by a pediatric doctor/ nurse (n agree with statement, %)	59/173 (34.1)	5/13 (38.5)	0.83
I received timely information; I had sufficient time to decide whether I wanted to vaccinate my child (n agree with statement, %)	139/173 (80.3)	8/16 (50.0)	**0.01**
Rotavirus vaccination should be available for all children (n, %)	94/171 (55.0)	9/13 (69.2)	0.32

Percentages are derived excluding respondents with missing information. Statistical significance (p-value <0.05) is highlighted in bold.

Discussion

This study evaluated the implementation of a targeted rotavirus vaccination program for infants with MRC in secondary and tertiary care. This strategy resulted in 52.3% of eligible infants receiving at least one dose of HRV (with a wide variety in coverage among sites and infants from different risk-groups). Less than 40% of parents were informed about the vaccine as part of standard care and reaching these vulnerable infants proved difficult. If vaccinated, however, timely vaccination was feasible for over 90% of these medical risk infants.

A targeted vaccine strategy is rare, only Croatia implemented a risk-group rotavirus vaccination program in 2011[21] and Spain introduced rotavirus vaccination for premature babies between 25 and 32 weeks of GA.[22] Arguments for a targeted vaccine strategy are to protect those at highest risk of severe disease, mortality and complications[16]. Vaccination coverage is known to be lower among premature infants <33 weeks of GA and LBW infants[23], as was also observed for rotavirus vaccination[24]. Yet, this was studied in a setting with universal rotavirus vaccination. Whether a targeted vaccination program was feasible in practice with sufficient coverage among these risk-group infants was not systematically assessed.

This implementation study indicates that a targeted rotavirus vaccination strategy for infants

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with MRC in secondary and tertiary care seems not satisfactory for several reasons. Even though information provision was perceived as clear by most of the survey respondents and the majority of HCP believed all eligible infants were routinely informed about rotavirus vaccination, less than 40% of parents stated that they received information on rotavirus vaccination as part of standard care. This was clarified by the interviews, suggesting that awareness among involved HCP was difficult to achieve. An on-site dedicated physician, as was available for the cohort-study participants, can offer individual parent counseling, facilitate the information provision and, assist in vaccine planning. This yielded a 87.3% vaccination coverage among the subset of infants participating in the cohort-study. Furthermore, recommendation by a HCP is known to be the main driver for choosing vaccination and 80% of parents would visit a HCP to gather information on rotavirus vaccination[25,26]. In addition, significantly fewer parents of unvaccinated infants felt they received information timely. Feeling overwhelmed by information, shortly before or at the time vaccination decisions have to be made, can lead to refusal[27,28]. In this survey parents and HCP of eligible infants were approached, perspectives of parents or HCP of ineligible infants on HRV vaccination we did not assess. A targeted vaccine program was chosen as preferred vaccination strategy by most survey respondents. However, for delivery of vaccines medical doctors in particular favored counseling and vaccine delivery by youth healthcare professionals at well-baby clinics, rather than at the hospital. Endorsement by medical doctors was brought forward as a facilitator during the interviews and suggests a need for collaboration with hospital care. As observed throughout the project by the interviewees, the varying out-patient follow-up policies resulted in individual based vaccine indications, making structured and uniform embedding into standard of care difficult. Only for infants with a GA < 30 weeks or birthweight below 1000 grams a national follow-up policy is available[29,30]. In addition, uniform recommendations about on-ward rotavirus vaccine administration and off-label use in those <27 weeks GA are needed for consistency in policy and across sites.

If a hospital-based targeted vaccination program for medical risk infants is the preferred strategy, we recommend an elaborate educational program for involved HCP as well as integration with the existing NIP structure, which – in the Netherlands - is executed by youth healthcare professionals.

In the small group of infants with GA <27 weeks treated in an off-label policy hospital we observed timely vaccination is possible in 86% and with an acceptable safety profile (**chapter 5**).

There were some limitations. First, although offering HRV as part of standard care to infants with MRC was suggested by the RIVAR protocol, it was not mandatory or structured in a single format. We therefore relied on each participating hospital how they incorporated this new standard of care and supervised execution of the program. As mentioned, we saw a broad variety in on-site protocols which possibly reflected in the achieved vaccination coverage. However in the absence of a national guideline, rotavirus vaccination should be adopted to local policy. Thus, our results reflect real life practice for targeted rotavirus vaccination.

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Secondly, within the study protocol the upper limit for vaccination with first dose was 14 weeks (which is earlier than according to the product information) and not all hospitals in the Netherlands participated. The window of opportunity for rotavirus vaccination was thereby narrow and limited to care in participating hospitals. The Dutch healthcare infrastructure has limited NICU and neonatal post-IC/HC beds, creating a relatively fast referral policy for medical risk infants to general pediatric wards. This might have reduced the amount of vaccinated infants. This limitation would be obsolete if rotavirus vaccination was not offered in a study specific setting.

Conclusions

To conclude, implementation of a targeted rotavirus vaccination strategy in secondary and tertiary care facilities in the Netherlands, yielded a suboptimal vaccination coverage among infants with MRC. Alternatively, implementation strategies including involvement of the existing NIP structure should be considered.

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Supplementary Material

Vaccination coverage for premature infants with gestational age (GA) < 27 weeks

Of 319 eligible infants with a gestation below 27 weeks, 81 received care in a participating hospital with a policy to vaccinate off-label. In total, 44 infants were male (54.3%), 13 (16.0%) were small for gestational age and one had a congenital pathology (1.2%). HRV was administered to 71 infants for at least one dose of HRV (87.7% 95%CI 80.3-95.0%). And 12 infants were vaccinated within six and nine weeks of postnatal age (16.9%), 61 before 14 weeks (85.9%).

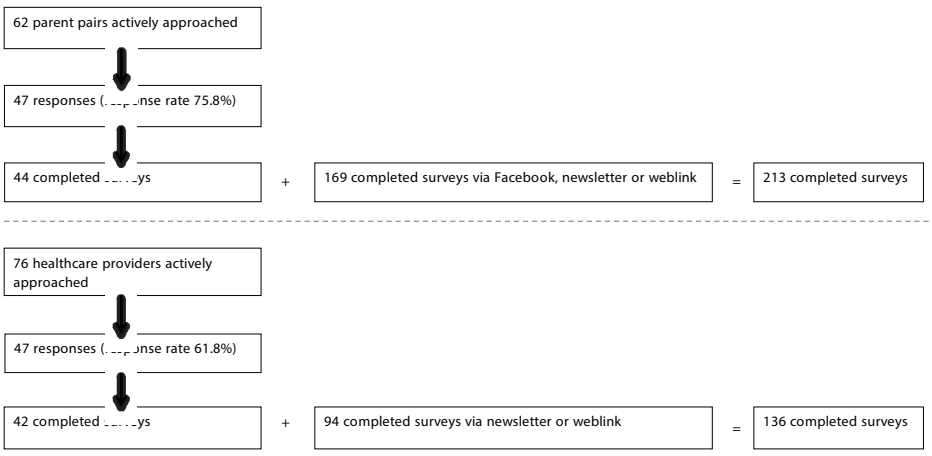

Figure S2 Flowchart of those approached for- and responded to the evaluation survey.

Study-specific outcomes of evaluation survey and in-depth interviews

For most HCP (53.1%), the RIVAR research personnel was the main source of information on rotavirus vaccination and the vaccination strategy in their hospital. As opposed to 46.9% that stated to be informed via colleagues, websites or other sources. Differences between medical doctors and other HCP are presented in **table S1**.

Table S1. Experience of the RIVAR study program among physicians

Statement	Medical doctors (N=51)	Other HCP (N=84)	p-value
I am aware of the rotavirus vaccination protocol within the RIVAR project (n agree with statement, %)	37/48 (77.1)	53/78 (67.9)	0.27

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Statement	Medical doctors (N=51)	Other HCP (N=84)	p-value
I know how to prescribe rotavirus vaccination for my patient within the RIVAR project (n agree with statement, %)	33/46 (71.7)	31/73 (42.5)	0.002
I know which agreements apply in my hospital about administration of rotavirus vaccination and how to arrange this (n agree with statement, %)	32/47 (68.1)	61/77 (79.2)	0.17

Percentages are derived excluding respondents with missing information. Statistical significance (p-value <0.05) is highlighted in bold.

The instructions for at home administration of the second dose were clear for 130 parents (74.7%) of vaccinated infants. Among the remaining 43 parents, 35 (81.4%) experienced some level of difficulty in administration of the second dose.

In-depth interviews: A study-specific infant related barrier is the eligibility criteria of care between six and 14 weeks of age in a participating facility. A pediatric nurse raised this during the interview: "*Half of this population is already at home before six weeks of age. Well baby clinics include follow-up for these children*.". A study specific healthcare related issue was the sole responsibility of pediatricians for implementation, two interviewees addressed this: "*Is it wise or useful to lay responsibility with the pediatrician? Shouldn't the well-baby clinic physicians function as a safety net? What is the benefit of making the pediatrician solely accountable? The only group that is missed by well-baby clinic physician are the infants with congenital disorders." and "The pediatrician is responsible for indication setting. But all practical aspects, administration and logistics of vaccination are not handled by them*.".

Another study-specific healthcare related barrier was confusion about RIVAR cohort-study and RIVAR project. A research nurse highlighted this during the interview: "*From the start of the project confusion existed with regards to cohort-study versus project. Most health care professionals in both hospitals initially perceived the rotavirus vaccine to be a study vaccine and as such only available to cohort-study participants. Since most eligible infants did not participate in the cohort-study it was thought that there was no need for involvement of the treating pediatrician.*".

A study specific issue that functioned both as barrier and facilitator was the use of a research nurse/employee to facilitate implementation: "*…Throughout the study period the research nurse was responsible, but therefore it was no standard care." and "The initial set-up was supported by sufficient time and money within the study budget. Then I found three medium care nurses who now administer the vaccination.* ".

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Implementation Evaluation Questionnaire for Parents

General

What is your gender?

a.Male

b.Female

How old are you?

How many children do you have?

What ethnic background do you have?

a.Dutch/Northern European

b.Surinamese

c.Antillean

d.Moroccan

e.Turkish

f.Other, i.e.

What is your highest level of completed education?

a.None completed education

b.Primary school

c.Lower vocational education

d.Secondary school

e.Secondary vocational education

f.High school

g.Higher professional education

h.Academic education (university/college)

Information provision about rotavirus vaccination and organization of administration

Rate the following six statements on a scale from 1 to 5.

1: totally disagree

2: disagree

3: neutral

4: agree

5: totally agree

I.I received enough information from the hospital about rotavirus vaccination.

II.The information provided to me about rotavirus vaccine, was easy to understand.

III.If I had questions about rotavirus vaccination, I knew who to address/I could contact.

IV.I received timely information about rotavirus vaccination; I had sufficient time to decide whether I wanted to vaccinate my child.

V.The instructions about vaccinating my child at home were clear to me.

VI.Giving my child the second dose of rotavirus vaccine at home was no problem for me.

VII.I was first informed about rotavirus vaccination by a pediatrician/pediatric resident or nurse.

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VIII.I searched for information about rotavirus vaccination on:

(Multiple answers possible)

a.RIVAR website

b.Website RIVM

c.Different websites, i.e.

d.RIVAR facebook group

e.Different facebook pages, i.e.

f.Other, i.e

g.I did not search for information

IX.Note, below, any suggestions to improve the provision of information about rotavirus vaccination.

X.For parents of unvaccinated infants; I choose not to vaccinate my child against rotavirus because…

The hospital where your child is (or was) receiving care joins the RIVAR project. Therefore your child belongs to the first group of children in the Netherlands who is offered rotavirus vaccination, without payment by parents/guardians. We are interested in your opinion about rotavirus vaccination for children in the Netherlands.

XI.Rotavirus vaccination should be available for

a.Children whose parents are willing to pay €135 for rotavirus vaccination, free market price.

b.Children with increased risk of rotavirus infection, such as children born premature, with low birth weight or congenital diseases.

c.All children (also without increased risk).

d.None of the above.

XII.My child could best be vaccinated against rotavirus by:

a.My childs general practioner

b.My childs pediatrician

c.The Public Health Service (GGD)/well baby clinic

d.Self-administration at home

Questions in grey are not asked to parents of unvaccinated infants.

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Implementation Evaluation Questionnaire for Healthcare providers

General

What is your gender?

a.Male

b.Female

How old are you?

What is your function?

a.Pediatrician/neonatologist

b.Pediatric resident

c.Pediatric or neontology nurse

d.Physician assistent

e.Other, i.e.

In your hospital rotavirus vaccination is implemented via the RIVAR project. This is a pilot where high risk infants (born premature and/or dysmature, and/or with a congenital disorder) are offered rotavirus vaccination, without costs involved.

Information provision about rotavirus vaccination and execution of the implementation program

Rate the following three statements on a scale from 1 to 5.

1: totally disagree

2: disagree

3: neutral

4: agree

5: totally agree

I.Information provision with regard to implementation of rotavirus vaccination in my hospital was clear.

II.I am aware of the rotavirus vaccination protocol within the RIVAR project.

III.In my hospital all eligible infants are routinely informed about rotavirus vaccination.

IV.I know how to prescribe rotavirus vaccination for my patient within the RIVAR project.

V.I know which agreements apply in my hospital about administration of rotavirus vaccination and how to arrange this.

VI.I am aware of the age restrictions for administration of rotavirus vaccination.

VII.In my hospital vaccination against rotavirus is sufficiently imbedded, all eligible infants routinely receive vaccination within the age restrictions according to the RIVAR project.

VIII.I have sufficient knowledge about the working mechanism of rotavirus vaccination.

IX.I have sufficient knowledge about the adverse events of rotavirus vaccination.

X.In my hospital parents are first informed about rotavirus vaccination by a pediatrician/pediatric resident or nurse.

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XI.I received information about rotavirus vaccination via:

(Multiple answers possible)

a.RIVAR implementation group-presentations

b.RIVAR website/e-learning

c.Website of RIVM

d.Other website, i.e.

e.RIVAR research dedicated physician

f.Colleagues

g.Other, i.e.

XII.Which hurdles do you encounter in the execution of the RIVAR implementation program to prevent eligible infants to receive rotavirus vaccination in your hospital?

(Multiple answers possible)

a.A child is already discharged and doesn't receive care within the age-restrictions for vaccination.

b.A child is too ill or instable to receive vaccination with the age-restrictions.

c.In my hospital children are not vaccinated on the ward, it regularly occurs that a child is hospitalized past 14 weeks of postnatal age.

d.It regularly happens that parents refuse rotavirus vaccination for their child.

e.Difficulties in prescribing rotavirus vaccination.

f.Other, i.e.

XIII.Note, below, any suggestions to improve the provision of information about or the execution of rotavirus vaccination implementation program.

The following questions concern your opinion about rotavirus vaccination for children in the Netherlands.

XIII.Rotavirus vaccination should be available for

a.All children, invested with the National Immunization Program.

b.Children with increased risk of rotavirus infection, such as children born premature, with low birth weight or congenital diseases, free of charge.

c.Children whose parents are willing to pay the free market price for vaccination.

XIV.If a, I am in favor of universal rotavirus vaccination because:

(Multiple answers possible)

a.Rotavirus infections causes high disease burden in children, even when they are otherwise "healthy".

b.The typical seasonality of rotavirus infections lead to capacity problems in pediatric hospital care.

c.The costs of vaccination are outweighed by the benefits in the form of disease reduction (cost effective).

d.It is complicated to implement a targeted vaccination program for high risk infants only, universal vaccination is more efficient.

e.Other, i.e.

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XV.If b, Rotavirus vaccination should be free of charge and part of standard care for high risk infants; born premature or dysmature or with congenital disorders. But not for "healthy" infants, because:

(Multiple answers possible)

a.In "healthy" infants the costs of vaccination dont outweigh the benefits, the money could be better spent.

b.In "healthy" infants, the severity and frequency of rotavirus infections is insufficient to justify vaccination.

c.Adding an extra vaccination to the National Immunization Program affects compliance.

d.Other, i.e.

XVI.If b, The responsibility for indicating eligible infants should lay with:

a.The preventative care system, the municipal health physicians set the indication.

b.The first line care system, the general practitioner sets the indication.

c.The second- and third line care system, the pediatrician sets the indication.

XVII.If b, Which healthcare system should deliver rotavirus vaccination to eligible infants:

a.Rotavirus vaccination administered via second and third line pediatric care (conform RIVAR project).

b.Rotavirus vaccination administered via second and third line pediatric care in cooperation with well-baby clinics.

c.Rotavirus vaccination administered via first line care, general practitioner.

d.Rotavirus vaccination administered via well-baby clinics, second and third line pediatric care not involved.

e.Other, i.e.

XVIII.If c, Rotavirus vaccination should not be implemented as standard of care in the Netherlands, because:

(Multiple answers possible)

a.The risk of adverse events (such as intussusception).

b.The rotavirus disease burden doesn't justify implementation.

c.The high costs related, the money could be better spent.

d.Other, i.e.

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Chapter 8

Summary and general discussion

Introduction

Rotavirus is a dominant cause of acute gastroenteritis (AGE) in children.[1,2] Rotavirus AGE is characterized by acute onset of diarrhea, fever and/or vomiting. Morbidity and mortality due to rotavirus disease in a developed country, like the Netherlands, primarily affects infants with prematurity, low birth weight or presence of a severe congenital disorder.[3] Providing rotavirus vaccination to this specific population was thought to prevent at least those infants most at risk of severe disease.[4]

This thesis described the Risk-group Infant Vaccination Against Rotavirus (RIVAR) project, in which rotavirus vaccination was provided to a specific medical risk population with the aim to study vaccine effectiveness, safety and program feasibility. The group of infants studied in the RIVAR project was defined as infants with 1) one or more medical risk conditions (MRC): premature birth (before 36 weeks of gestation), a low birthweight (below 2500 grams) and/or, a severe congenital pathology. And 2) receiving prolonged in- or out-patient care (between six and 14 weeks of postnatal age) in a participating hospital. This chapter includes a summary of most important findings, a discussion of these findings and recommendations for clinical practice and future research on infant rotavirus vaccination.

Summary of this thesis

As a baseline measurement and to guide prioritization for prevention strategies we assessed the community burden of all-cause AGE, and pathogen specific disease in this population, using data from the unvaccinated RIVAR cohort (**chapter 2**). Community disease burden due to rotavirus was not systematically studied previously, and prior information on rotavirus infections in this medical risk population was obtained from studies conducted in hospital settings. We found that the incidence of all-cause AGE in medical risk infants was comparable to that among healthy infants, however severity in terms of symptoms, healthcare attendance and hospitalization was two to three times higher. Rotavirus and norovirus were most frequently detected as pathogen, and rotavirus resulted in a more severe disease course. In addition, the all-cause AGE in this population led to important societal impact as reflected by 30% daycare absenteeism and parental work loss. We concluded that, compared to studies in healthy infants, the community AGE disease burden among medical risk infants was considerably increased.

Next, we updated a previous cost-effectiveness analysis[4] of rotavirus vaccination to take into account the change in rotavirus epidemiology, lower hospitalization rates and updated estimates on community disease burden (**chapter 3**). We estimated that universal rotavirus vaccination would generate the highest reduction in population disease burden due to rotavirus. However, targeted vaccination (of medical risk infants) was cost-saving in the main and sensitivity analysis,

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and had the most favorable risk-benefit profile with regard to intussusception. Based on these results, selective vaccination against rotavirus AGE can be favorable on all criteria, provided that the vaccine is effective in a population with MRC.

We subsequently evaluated whether vaccination indeed offered protection against rotavirus disease in RIVAR eligible infants. Based on efficacy trials among healthy term and preterm infants, a conservative vaccine effectiveness of 60-80% was anticipated. In the RIVAR project the human rotavirus vaccine (HRV, Rotarix, GSK Biological SA, Belgium) was used. By comparing the vaccinated versus unvaccinated RIVAR cohort we were able to assess rotavirus vaccine effectiveness among MRC infants (**chapter 4**). In contrast to the vaccine efficacy trials, HRV was not significantly protective against severe rotavirus AGE (vaccine effectiveness 30%; 95%CI -36;65%) in this study, and no impact on rotavirus hospitalizations was observed. As the numbers were however small, a post-hoc analysis was added for all-cause AGE and rotavirus AGE of any severity, but comparable and non–significant effects were observed, suggesting that rotavirus vaccine performance is minimal in infants with MRC. The upper 95% confidence limit of rotavirus vaccine effectiveness was 65% in our study, as opposed to a mean estimate of 90% for healthy infants in high-income settings.[5,6] In addition, lower 95% bound vaccine effectiveness estimates for healthy infants are 76-84%.[5,6] We therefore conclude that HRV effectiveness is significantly lower in MRC infants and this finding signifies the need for risk-group specific research.

Moreover, we found that serious adverse events that were possible vaccine reactions[7] occurred more frequently than previously described in the population with MRC, at a rate of 0.25 per 100 vaccine doses (**chapter 5**). The majority of adverse events were gastrointestinal. Possible causality was based on the temporal relationship with HRV administration and biological plausibility in terms of their pathophysiology. Overall, more (gastrointestinal) adverse events were reported among vaccinated premature infants as opposed to non-rotavirus vaccinated (RR 1.07, 95%CI 1.04;1.10). In the group of infants with a gestational age below 27 weeks, who were vaccinated off-label, rotavirus vaccination was generally well tolerated and no safety signals were observed. Acknowledging the combined effectiveness and safety results, clinicians should weigh the risks and benefits of rotavirus vaccination for the individual infant with MRC.

Furthermore, there was no evidence of benefit due to non-specific effects of rotavirus vaccination (**chapter 6**). Live-attenuated vaccines, like HRV, have been associated with beneficial non-specific effects. This phenomenon is attributed to trained immunity, a vaccine induced modification of the innate immune system that reduces the risk and/or severity of disease by non-target infections. In our study, HRV was not protective against acute hospitalization when excluding admissions for AGE (hazard ratio: 0.91, 95%CI 0.76;1.16). In addition, we did not

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observe a reduction in cumulative days hospitalized or incidence of acute respiratory illness, as would be expected on the basis of trained immunity mechanisms.

Finally, the implementation strategy used for the RIVAR project yielded a vaccination coverage of 52% (95%CI 51;54%) with a wide variation in coverage between infants with different MRCs and between hospitals (**chapter 7**). In the Netherlands, routine childhood vaccinations are delivered via youth health care professionals in well-baby clinics. Setting the indication for, and administering rotavirus vaccination selectively to medical risk infants required a different infrastructure. We therefore implemented this targeted vaccination program in secondary and tertiary pediatric medical care centers. Based on evaluation surveys among parents and health care professionals (HCP), and in-depth interviews with involved HCP we provided suggestions for improvement of a hospital-based targeted rotavirus vaccination program for medical risk infants. This included an elaborate educational program with a dedicated on-site physician to raise awareness, nationally implemented recommendations to avoid too much variability in local hospital policies and, dependent on the existing local immunization structure and setting, involvement of youth HCP for counseling and vaccine delivery.

General discussion

The decision making process on rotavirus vaccination in the Netherlands has been a long and bumpy road. What we have learned throughout the RIVAR project and from its results is ultimately reflected in the reversed ministerial decree on implementation of rotavirus vaccination, and a renewed request to the Dutch Health Council to revise its advisory statement.[8]

The first rotavirus vaccination advisory statement, years before the RIVAR study started, dated from 2007 and concluded that there was insufficient evidence for the added value of universal rotavirus vaccination, given unknowns about genotype epidemiology and cost-effectiveness in the Netherlands.[9] In 2013 rotavirus vaccination was again reviewed by the Health Council, however no consensus was reached and a formal report was never published.[10,11] In 2017, the Health Council advised to implement rotavirus vaccination, at least for infants with MRC.[12] A year later, the Ministry of Health decided to implement a selective rotavirus vaccination program by 2019, which was later postponed to 2020.[13,14] While preparations were in progress, the preliminary results of the RIVAR project were communicated to the National Institute of Public Health and the Ministry of Health. This led to the decision on 30 April 2020 to cancel the implementation and to request an updated advice from the Health Council, incorporating lessons learned from the RIVAR project.[8,15] At the time of writing of this discussion, the new advisory statement is pending and no rotavirus vaccination program is implemented in the Netherlands, contrary to the situation in over 100 countries worldwide (**figure 1**).[16]

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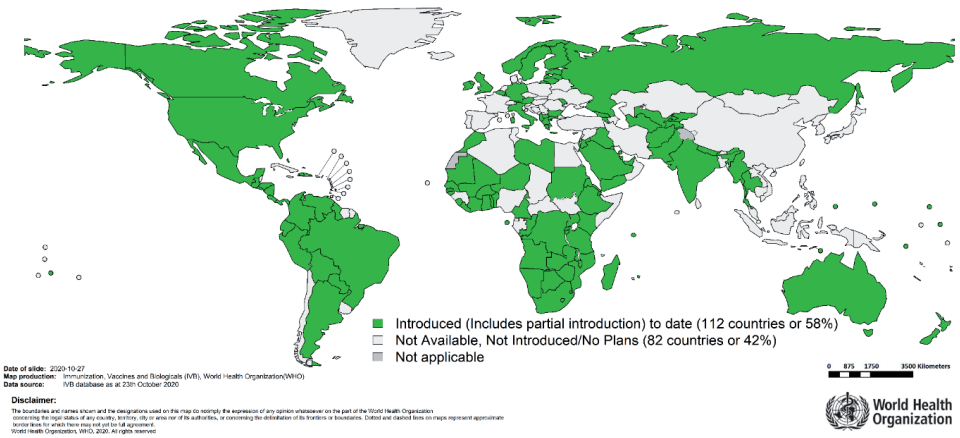

Figure 1. Rotavirus vaccine global introduction status. 2020 @WHO.

In the following paragraphs the lessons learned from the RIVAR project and its results will be discussed, related to previous literature and translated into future (clinical and research) directions.

Vaccine effectiveness

The most remarkable finding of this thesis is the low rotavirus vaccine effectiveness for infants with MRC. The results of this study appear in sharp contrast to results from previous (pre-licensure) efficacy studies in (subgroups of) medical risk populations. Importantly, efficacy studies (randomized controlled trials) are usually performed in highly selected populations under strictly controlled conditions, whereas in daily practice the intervention applies to more heterogeneous populations. The RIVAR project was specifically designed as an observational phase IV (post-licensure) effectiveness study that is suited to provide real world evidence for a heterogeneous, high-risk population. Where traditional observational study designs may suffer from bias due to confounding by indication, the quasi-experimental design of the RIVAR study was chosen to appropriately avoid this. Therefore, these results are considered representative and generalizable.

Differences between the current study and prior "positive" effectiveness studies in medical risk populations are presented in **table 1**.

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Table 1. Differences in study -procedures, -population and -period between RIVAR and prior positive rotavirus vaccine effectiveness studies

	RIVAR	IVANHOE[17]	Dahl et al[18]
Study procedures			
Design	Before-after cohort	Active hospital based surveillance	Longitudinal administrative data
Data collection	Prospective	Prospective	Retrospective
Vaccine	Rotarix	RotaTeq	RotaTeq and Rotarix
Exposure	At least one dose	Vaccine introduction	Receipt of at least one dose rotavirus vaccination
Adjustment	Seasonal variation by rotavirus detections from weekly sentinel surveillance	Variation in rotavirus epidemiology by using an unvaccinated population	Seasonal trends by birth month-year in annual and peak season
Statistical analysis	Cox regression	Poisson regression	Poisson regression
Outcome	Severe rotavirus AGE	Rotavirus hospitalization	Rotavirus hospitalization
Study population			
Inclusion criteria	Premature infants 24-36+6 weeks Low birthweight Congenital disorders Or combination of all above	Premature infants 25-36+6	(very) Low birthweight <1500 or <2500 grams
Gestational age	Mean (SD): 32+5 weeks+days (3+5)	Median (range): 34.7 weeks (30.2-35.6)	n/a
Birthweight	Median (IQR): 1830 g (822-2838)	Median (range): 2320 g (1575-2670)	n/a
Number of participants	1482	217	78,288
Vaccination coverage	84.5%	41.9-45.2%	64-82%
Outcome events	38	27	n/a
Main outcome estimate	30% (95%CI -36;65%)	62% (95%CI 45;73%)	90-100% (95%CI n/a)
Study period			
Time	2014-2019	2002-2010	2001-2015
Country	The Netherlands	France	North-America
Circulating rotavirus strains	G2P4, G3P8, G9P8, G4P8[19–21]	G1P8, G9P8[22]	G1P8, G3P8[23], G12P8[24,25]
Universal rotavirus vaccination	No	Within study area, from 2007 onwards	Yes, from 2006 onwards

Abbreviations: AGE= acute gastroenteritis, SD = standard deviation, n/a = not available, IQR = interquartile range, CI = confidence interval.

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Changing rotavirus epidemiology

One of the possible explanations for the low vaccine effectiveness is the change in rotavirus epidemiology. Globally, the introduction of rotavirus vaccines has substantially changed the epidemiology.[26–28] Furthermore, in the Netherlands and other non-vaccinating countries epidemiology of rotavirus was altered as well. In general, rotavirus epidemiology is influenced by vaccination, climate and temperature changes, birthrates, previous rotavirus seasons and size of susceptible populations.[29–31] Changes observed in recent years in both rotavirus vaccinating and non-vaccinating countries include a shift from annual high endemic peaks towards biennial, lower and later peaks.[28,32] Strain diversity changed from primarily G1P8 genotype dominance, to alternating high prevalence of G2P4, G3P8, G4P8 and G9P8 in the more recent years.[19–21] This change in circulating genotypes is thought to be the result of vaccine-induced selective pressure.[33]

Rotavirus vaccination is less well effective towards (partly) heterotypic strains, that were circulating more after 2014. During the RIVAR study period less than 14% of rotavirus detections by national surveillance sentinel laboratories were tested as G1P8 strain, while the fully heterotypic G2P4 strain became more prevalent with 12%.[19,34,35] This altered landscape of rotavirus strain distribution may have contributed to our low vaccine effectiveness estimates (**table 1**). While early on, rotavirus vaccine effectiveness estimates against partly heterotypic strains were 74-87% and 85-94% against fully heterotypic strains (compared to 89-95% against homotypic strains).[36–38] Recent estimates from the era of altered strain diversity are not available. In general, there is a lack of research on rotavirus vaccine performance or effectiveness in more recent years. Within five years after the first rotavirus vaccines were globally introduced, 24 primary studies reported estimates of vaccine effectiveness in different geographic regions. In the window between five to ten years after vaccine introduction only eight studies reported on vaccine effectiveness and no studies have yet been published that cover the period beyond ten years post-introduction.

However, in light of the substantial changes in rotavirus strain distribution, continued monitoring of vaccine effectiveness is warranted. For influenza, a European Influenza Surveillance Scheme (EISS) exists. Strain-specific and overall vaccine effectiveness reports are published for each influenza season based on the available clinical and epidemiological data.[39–41] This aids in monitoring the virus, mismatch with vaccine strains and circulating strains in Europe. For rotavirus, the National Respiratory and Enteric Virus Surveillance System (NREVSS) and New Vaccine Surveillance Network (NVSN) in North America provided reports on clinical and epidemiological data of rotavirus until 2016.[25,42] In Europe, EuroRotanet (a network of collaborating laboratories)[43] already collects information on rotavirus types co-circulating in Europe. Integration of these data in vaccine performance studies seems feasible. Active surveillance of rotavirus epidemiology should be combined with monitoring vaccine effectiveness to better understand the impact of strain replacement on vaccine performance and burden of disease.

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Infant population characteristics

Another possible explanation for the lower vaccine effectiveness is related to the study population. Some host-factors are known to influence the rotavirus vaccine immune response, including gut dysbiosis, genetic factors and intestinal coinfections. While these associations have mainly been established based on studies in developing countries,[44] they may also be relevant for infants with MRC. For instance, gut dysbiosis may also exist in medical risk infants because of prematurity, antibiotic treatment or critical illness. Microbiome composition is additionally thought to differ from term infants, depending on gestational age. These differences, also in microbiota with positive effects on immune training, were described to exist at six weeks of age (the time point of first rotavirus vaccination).[45] In addition, premature infants have impaired functioning of the mucosal barrier, and innate and adaptive immune system.[46] This may contribute to decreased effectiveness of mucosal vaccines in premature infants as was demonstrated for oral polio vaccines.[47,48] Furthermore, infants with congenital cardiovascular pathologies, that comprised the largest group of congenital disorders in our study population, are known to be immunocompromised pre- and post- cardiac bypass surgery, or may suffer from genetic disorders affecting the immune system and consequently rotavirus vaccine immune response. Serum anti-rotavirus IgA testing, as was performed in the pre-licensure clinical efficacy trials,[49] and their geometric mean titer would add valuable information to assess the immune responses in infants with MRC.

In summary, the RIVAR study population included more vulnerable infants with regard to gestational age, birthweight and comorbidities, compared to other studies among specific risk populations (**table 1**), which may explain the lower vaccine effectiveness. In addition, these studies were performed in settings with universal rotavirus vaccination, where herd protection potentially contributed to measured vaccine effects.

To improve rotavirus vaccine performance among medical risk infants, alternative vaccination strategies should be explored. The novel parenteral rotavirus vaccines may offer a solution.[50] Vaccine responses to other parenteral childhood vaccinations were adequate in a subset of the prematurely born infants participating in RIVAR.[51] Alternatively, the rotavirus vaccination schedule could be adapted to improve immune responses, for instance by adding a third dose for Rotarix or by changing intervals between dosing.

Non-specific effects of vaccination

For live-attenuated vaccines, some studies have reported beneficial non-specific effects. Trained immunity is the proposed mechanism by which live-attenuated vaccines enhance the innate immune response to subsequent and non-target infections.[52,53] The RIVAR study population would greatly benefit from such non-specific effects, as these infants are at increased risk of respiratory infections and hospitalizations. However, we did not find significant beneficial effects on hospitalization for non-target diseases or on incidence of acute respiratory infections after

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rotavirus vaccination in medical risk infants. So far, inconclusive evidence is reported on the overall benefit of (live-attenuated) childhood vaccines on non-target diseases[54,55] and the mechanisms that could mediate the non-specific effects have not yet been fully elucidated. Therefore, further evidence is needed before these effects could be considered for inclusion in cost-benefit analyses. For rotavirus, the combined data from the rotavirus vaccine phase III efficacy trials analyzed with non-AGE acute hospitalization or hospitalization for respiratory infection as outcome, should be suitable to provide more definite answers on the protection of rotavirus vaccination against non-target diseases.

Hospital-based targeted vaccination

The hospital-based targeted rotavirus vaccination strategy resulted in suboptimal overall vaccination coverage and several hurdles were encountered as identified in the survey and in-depth interviews. Parents of medical risk infants were insufficiently reached and providing quality of care by timely and personal vaccine counseling proved difficult to achieve for all eligible infants. Among the cohort-study participants however, better counseling was feasible with the aid of a dedicated RIVAR researcher and resulted in a much higher vaccination coverage of 85%. It is however unlikely that this level of support could be maintained in a national roll-out of this hospital-based vaccination strategy. At the time of writing, only two countries, Croatia and Spain, have implemented targeted vaccine programs for rotavirus vaccination.[56,57] No estimates on vaccination coverage or effectiveness from these countries have been published.

Alternative strategies to deliver rotavirus vaccination to medical risk infants therefore need to be considered. In the Netherlands the national immunization program is invested with well-baby clinics and executed by youth HCP. Using this infrastructure for rotavirus vaccination of infants with MRC is possible. However, due to (prolonged) hospital stay and frequent post-discharge outpatient visits, many MRC infants do not receive care by youth HCP within the tight age restrictions of first dose administration of rotavirus vaccine. Therefore, some kind of cooperation between youth HCP and hospital care will be necessary, which already proved difficult to implement in practice.[14]

Implications for vaccine policy and future research

The two main aims of the RIVAR project were to assess feasibility and effectiveness of a selective rotavirus vaccination strategy targeting infants with MRC. The achieved reduction of severe rotavirus acute gastroenteritis was limited and the feasibility of selective hospital-based vaccination proved suboptimal. In this paragraph, I will weigh protective effects (direct, indirect and non-specific), safety profile, different vaccination strategies and cost-effectiveness in order to arrive at recommendations for future rotavirus vaccination policy for medical risk infants.

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Overall benefit of rotavirus vaccination for medical risk infants

Rotavirus vaccination for infants with MRC in this study did not result in a significant reduction in hospitalization due to rotavirus AGE, although a 30% reduction (the point estimate of our study) could have some clinical relevance. In addition, no difference in incidence of rotavirus AGE of any severity was observed between vaccinated and unvaccinated infants. Vaccination coverage using a hospital-based targeted vaccination strategy reached 52%. Therefore, the assumptions on vaccination effectiveness and coverage in the updated cost-effectiveness analysis (**chapter 3**) no longer apply. It is highly unlikely that targeted rotavirus vaccination would be cost-effective from either a healthcare or societal perspective at the free market price given the vaccine effectiveness estimate of 30%. Targeted vaccination could be more favorable if reductions in hospitalizations could be achieved through non-specific effects. However, such non-specific beneficial effects of rotavirus vaccination could not be demonstrated in our study.

Safety and tolerability profile

Some safety signals were detected in our study and have also been described earlier.[58] An evaluation of reports from the vaccine adverse event reporting systems in America and Europe covering ten years, concluded that most adverse events following rotavirus vaccination were gastrointestinal, non-serious and were already included in the Summary of Product Characteristics (SPCs), like vomiting and diarrhea (n>1000). Respiratory tract infections, Kawasaki disease and neurological disorders were also reported, although causality is difficult to establish and the authors recommend further investigation. The reported odds ratios, for rotavirus vaccines compared to other vaccines, were 8.8, 14.6 and 2.3 for respiratory tract, Kawasaki and neurological diseases respectively. These passive adverse event surveillance databases do not include information on patient characteristics (such as presence of MRC) and stratified risk assessment for comparison was therefore not possible. Our tolerability results also showed most adverse events were gastrointestinal and non-serious (i.e. did not require healthcare attendance). Yet the few severe (mainly gastrointestinal) adverse events we observed cannot be ignored.

Risk-benefit ratios

The combined results on vaccine effectiveness, overall health benefits and safety risks of rotavirus vaccination for infants with MRC warrant recalculation and weighing of the risk-benefit ratios. If we conservatively consider only those serious adverse events (SAE) for which a link with other routine national immunization program vaccines is unlikely given the nature of events (gastrointestinal), there were eight vaccine related SAE among 2077 vaccinated infants (described in **chapter 5**). This translates to a population attributable risk of vaccine related SAE after rotavirus vaccination of 0.4%. Which means that four in 1000 vaccinated

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MRC infants experience a SAE. The potential benefit of vaccination we calculate based on a 30% vaccine effectiveness and a 7.6% incidence of severe rotavirus AGE up to 18 months of age (derived from rotavirus incidence of 14.6 per 100 person years and 43% severe disease in the unvaccinated population as described in **chapter 2**). This leads to a 2.3% population attributable risk reduction of severe rotavirus AGE by vaccination in medical risk infants. Jointly, these calculations result in a risk-benefit ratio of 1:6, meaning that per induced SAE six severe rotavirus AGE cases were prevented. In this comparison of risks and benefits we ignored all non-serious adverse events, that occur at one in 10 rotavirus vaccinated infants. Our previous analyses which assumed over 80% vaccine effectiveness and higher AGE incidence,[4] described in **chapter 3**, resulted in a far more favorable risk-benefit ratio of targeted vaccination.

Some further discussion is needed on infants born after 32 weeks of gestation and term infants with congenital disorders. Within RIVAR the focus was on extreme vulnerable medical risk infants with prolonged care, i.e. infants should still receive care at time of first dose rotavirus vaccination. Consequently, our results cannot be extrapolated to healthy and stable premature infants without prolonged postnatal care. This includes mostly infants born after 32 weeks of gestation. For moderately premature infants it might be best to rely on the prior effectiveness studies (**table 1**) and pre-licensure trials of Omenaca and Goveia et al.[59,60] In these trials, 303 and 2070 premature infants were studied for immunogenicity and efficacy of rotavirus vaccination respectively. Only a small proportion had a gestational age below 32 weeks and none had comorbidities. IgA seroconversion rate after two doses of Rotarix was 85.7% (95%CI 79.0;90.9%)[59] and efficacy against rotavirus AGE of any severity was 73% (95%CI -2.2;95.5%) after three doses of RotaTeq.[60]

Another group that could benefit more from rotavirus vaccination includes the subgroup within RIVAR of term infants with congenital disorders. The point estimate for vaccine effectiveness in this group was slightly higher (hazard ratio: 0.51). However, due to fewer participants in the subgroups the estimates are less precise. For these infants, evidence from the post-licensure studies of Javid and Fang et al should also be considered.[61,62] These two studies showed sufficient seroconversion and an acceptable safety profile of rotavirus vaccination for infants with intestinal disorders or failure.

Vaccination strategy

Based on the RIVAR study, vaccination of infants with MRC is no longer cost-effective and the estimated risk-benefit ratio gives reason for concern. In order to protect these infants from rotavirus disease, an alternative vaccination strategy should be considered. Routine universal rotavirus vaccination or universal mass vaccination, as 107 countries worldwide have implemented,[16] could be such a strategy. With universal rotavirus vaccination included in the national immunization program at well-baby clinics, indirect effectiveness (herd effect)

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could protect medical risk infants. Herd effect is achieved via decreased transmission in the population if a sufficient fraction has been vaccinated. This prevents disease occurring among unvaccinated individuals, and occurs in a setting with universal vaccination. Estimates of herd effect from universal rotavirus vaccination varied between two and 77% per season in a North-American study,[28] a meta-analysis reported 48% (95%CI 39;55%) overall indirect effectiveness among children below five years of age.[63] In addition, the population with underlying medical conditions or perinatal morbidity is mostly affected by nosocomial rotavirus infection early in life.[3] Due to frequent hospitalization and prolonged admission duration, their risk of in-hospital rotavirus acquisition increases. Universal rotavirus vaccination was reported to reduce 72% of nosocomial rotavirus infections per year compared to no vaccination.[64]

For future research, I propose that the following five suggestions are taken into consideration:

a) The (changing) epidemiology of rotavirus. Two studies underlined the need for studying rotavirus strain diversity and continuation of genotype surveillance.[29,65] Surveillance of rotavirus epidemiology and corresponding vaccine effectiveness is advised in order to monitor changes in circulating strains and its effect on vaccine performance.

b) Vaccine performance should be separately assessed for specific medical risk populations as evidence from general or trial populations may not necessarily be generalizable. This thesis demonstrated that separate studies in specific at-risk populations can provide valuable information in order to guide policy on vaccine administration, programs and implementation.

c) Given the poor vaccine effectiveness in our study population, alternative types of rotavirus vaccines, which are currently still in development (**figure 2**), should be considered.[50] Parental subunit vaccines or inactivated vaccines could improve immune responses in medical risk infants and hopefully deliver sufficient protection.

d) The microbiome association with rotavirus immune response. This should be verified in infants as there are several hypotheses from animal and human studies that immunity is influenced by the gut microbiota.[66–69] The microbiota is a potential stimulant,[45] and a potential target for interventions to promote the immune response needed for (mucosal) rotavirus vaccination in infants with MRC.

e) Rotavirus serum IgA levels in medical risk infants after vaccination. Serum IgA antibody titer is considered the best correlate of protection, and rotavirus vaccine performance as reported by a meta-analysis of efficacy trials.[49] A lower rotavirus serum IgA titer in our population would corroborate the hypothesis of an altered immune response in infants with MRC.

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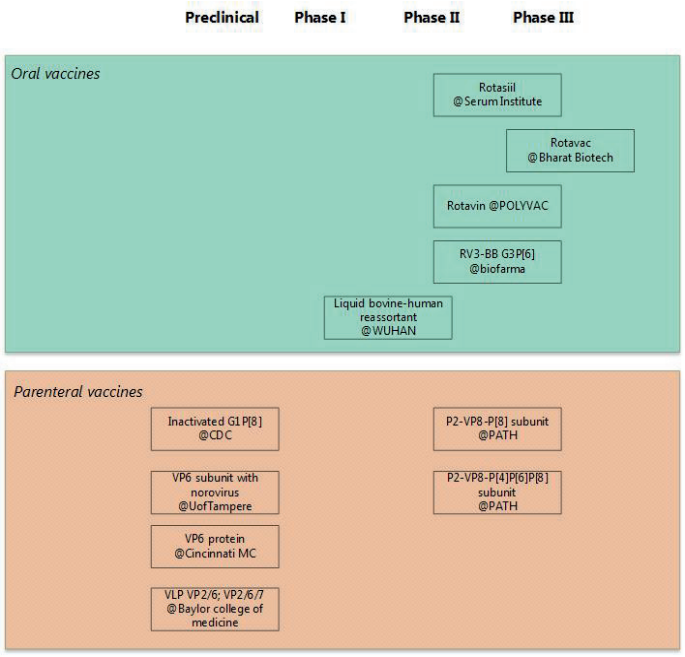

Figure 2 Rotavirus vaccine development pipeline. Based on Burke et al - *Curr Opin Infect Dis* 2019

In the RIVAR study the full safety-effectiveness-implementation spectrum of rotavirus vaccination for infants with MRC was evaluated. Therefore, for this specific patient population, guided recommendations can be made. Non-specific effects of rotavirus vaccination are ignored in this recommendation, because these could not be confirmed in our study but we acknowledge that further research is required. Considering the combined (cost-) effectiveness, safety and feasibility results I would advise a universal rotavirus vaccination strategy excluding premature infants below 32 weeks of gestation and premature infants with comorbidities. At high vaccination coverage, this strategy could yield sufficient indirect effectiveness (herd effect), and reduction in nosocomial infections for the extreme vulnerable medical risk infants, as well as substantial reductions in rotavirus disease in the total infant population, resulting in cost and healthcare benefit. The existing national immunization program infrastructure can be used, guaranteeing quality of parent counseling and high uptake in the population. Because the most extremely vulnerable infants with MRC are excluded, administration within age restrictions of rotavirus vaccination will generally be feasible at well-baby clinics. Rotavirus vaccine effectiveness in healthy premature and term infants with congenital (gastrointestinal) pathology has been studied satisfactory, even though newer studies in the current epidemiology landscape are needed.

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Summarizing conclusion

Rotavirus vaccine (cost-) effectiveness and its safety profile among infants with medical risk conditions (MRC) and the implementation of a targeted rotavirus vaccination program have been studied and evaluated in this thesis. This population of infants with MRC should be prioritized for prevention since their disease burden is highest and substantial, even in a well-developed country like the Netherlands. However, a hospital-based vaccination program targeted exclusively towards these medical risk infants showed limited protection and efficiency. Vaccine performance is subject to host characteristics and changing epidemiological conditions, which warrants population specific research and continued surveillance of vaccine effectiveness. The limited protection from current oral rotavirus vaccines among infants with MRC emphasize the need for alternative and optimized preventive strategies for rotavirus disease.

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Appendices A

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Dutch summary

Nederlandse samenvatting

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Introductie

Rotavirus is een belangrijke oorzaak van acute gastro-enteritis (AGE) bij kinderen. Rotavirus AGE kenmerkt zich door acuut ontstaan van diarree, koorts en/of overgeven. De ziektelast is hoog, vooral vanwege dehydratie, dat vooral bij zuigelingen kan optreden. In Nederland is het rotavirus verantwoordelijk voor 1900-3400 ziekenhuisopnames per jaar en vijf tot zes doden op kinderleeftijd. Deze ziektelast wordt in ontwikkelde landen, zoals Nederland, vooral ervaren door kinderen die te vroeg of te klein zijn geboren of kinderen met een aangeboren afwijking. Kinderen met een van deze drie medische risico aandoeningen hebben een verhoogd risico op ernstige complicaties als gevolg van rotavirus AGE.

Sinds 2006 zijn er werkzame, levend verzwakte orale vaccins tegen rotavirus beschikbaar voor zuigelingen, RotaTeq en Rotarix. Deze vaccins verminderen het risico op ernstige rotavirus AGE met meer dan 80%. De vaccins worden over het algemeen goed verdragen. Er is echter een klein verhoogd risico op invaginatie (een acuut ziektebeeld waarbij een deel van de darmen in elkaar schuift) in de eerste zeven dagen na de eerste dosis. Het risico op invaginatie wordt geschat op 1.1 tot 2.7 per 100.000 rotavirus gevaccineerde kinderen. De Wereldgezondheidsorganisatie (WHO) adviseert universele vaccinatie tegen rotavirus voor zuigelingen, vanwege de gunstige risico-batenverhouding van vaccinatie. In Nederland is er tot op heden geen rotavirusvaccinatie programma, in tegenstelling tot de situatie in meer dan 100 landen wereldwijd. De Gezondheidsraad heeft in september 2017 een advies uitgebracht waarin zij positief staat ten opzichte van universele vaccinatie en het rotavirusvaccin in ieder geval adviseert voor kinderen met een medische risicofactor. De minister van Volksgezondheid besloot in 2018 een gericht rotavirusvaccinatie programma te implementeren voor deze groep kwetsbare kinderen. Dit programma zou in juni 2020 starten. Echter, op basis van de voorlopige resultaten van dit proefschrift, werd in april 2020 de implementatie voor onbepaalde tijd uitgesteld en werd de Gezondheidsraad opnieuw om advies gevraagd over rotavirusvaccinatie, met inachtneming van de onderzoeksgegevens zoals beschreven in dit proefschrift.

In dit proefschrift wordt het Risk-group Infant Vaccination Against Rotavirus (RIVAR) project beschreven. In dit project werd rotavirusvaccinatie aangeboden aan zuigelingen met medische risico aandoeningen met als doel de vaccin effectiviteit, veiligheid en haalbaarheid van dit programma te bestuderen. De groep zuigelingen die in het RIVAR project werd onderzocht was gedefinieerd als zuigelingen met 1) een of meer medische risico aandoeningen: prematuriteit (geboren voor 36 weken zwangerschapsduur), een laag geboortegewicht (minder dan 2500 gram) en/of, een ernstige aangeboren afwijking. En 2) ontvangen van (poli-) klinische zorg (tussen zes en 14 weken postnatale leeftijd) in een deelnemend ziekenhuis. In totaal namen dertien ziekenhuizen op 15 locaties deel aan het RIVAR project. Het RIVAR project was ontworpen om twee hoofdvragen te beantwoorden en bestond uit de implementatie van een

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rotavirusvaccinatie programma gecombineerd met een voor-na cohort studie. De eerste vraag was, wat is de vaccin effectiviteit van rotavirusvaccinatie bij zuigelingen met een medische risico aandoening? En de tweede, is een gericht rotavirusvaccinatie programma in de tweede- en derdelijnszorg haalbaar? Als secundaire doelen werden de vaccin veiligheid en potentiele niet-specifieke effecten van rotavirusvaccinatie bepaald. Deze vragen werden beantwoord, nadat eerst de ziektelast van rotavirus in de samenleving werd geschat voor deze populatie kinderen met een medische risicofactor en de kosten-effectiviteitsanalyse werd herzien.

Samenvatting van het onderzoek in dit proefschrift

Om een uitgangsmeting te hebben en te kunnen prioriteren voor preventie strategieën, beoordeelden we de ziektelast van acute gastro-enteritis (AGE), en pathogeen specifieke AGE, voor zowel episoden die thuis plaatsvonden als waarvoor een arts werd geraadpleegd. Hiertoe gebruikten we de data van het rotavirus ongevaccineerde RIVAR cohort (**hoofdstuk 2**). De ziektelast in de samenleving door rotavirus was niet eerder systematisch onderzocht, en beschikbare informatie over rotavirusinfecties in de populatie kinderen met medische risico aandoeningen was verkregen uit studies in een ziekenhuis omgeving. We vonden dat de incidentie van AGE onder kinderen met een medische risicofactor gelijk was als voor gezonde kinderen, echter de ernst met betrekking tot symptomen, zorggebruik en ziekenhuisopname was twee tot drie keer verhoogd. Rotavirus en norovirus waren de meest gedetecteerde pathogenen, en rotavirus veroorzaakte het ernstigste ziektebeloop. Daarbij komt dat AGE in deze populatie leidde tot belangrijk maatschappelijke impact, weerspiegeld in 30% absentie van kinderopvang en ouderlijk werkverlies/verzuim. We concludeerden dat, vergeleken met studies in gezonde kinderen, de AGE ziektelast in de samenleving voor medisch risico kinderen aanzienlijk verhoogd was.

Vervolgens hebben we de bestaande kosten-effectiviteitsanalyse van rotavirusvaccinatie in Nederland opnieuw berekend, waarbij de verandering in rotavirus epidemiologie, de lagere aantallen ziekenhuisopnamen en de nieuwe schattingen van ziektelast werden meegenomen (**hoofdstuk 3**). Naar schatting zou universele rotavirusvaccinatie de grootste reductie geven in rotavirus ziektelast voor de gehele populatie. Echter, gerichte vaccinatie van zuigelingen met een medische risico aandoening was kostenbesparend in de primaire en sensitiviteitsanalyses, en had het gunstigste risico-baten profiel met betrekking tot invaginatie. Gebaseerd op deze resultaten zou gerichte vaccinatie tegen rotavirus AGE gunstig zijn op alle criteria, mits het vaccin effectief is in een populatie met een medische risicofactor.

Aansluitend evalueerden we of het vaccin inderdaad bescherming bood tegen rotavirusziekte in zuigelingen met een medische risicofactor. Op basis van eerdere schattingen over vaccin

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werkzaamheid onder gezonde a terme en prematuur geboren zuigelingen, werd een conservatieve vaccineffectiviteit van 60-80% verwacht. In het RIVAR project werd het humaan rotavirus vaccin (HRV, Rotarix, GSK Biological SA, België) gebruikt. Door de gevaccineerde en ongevaccineerde cohort deelnemers aan de RIVAR studie onderling te vergelijken konden we de rotavirusvaccin effectiviteit voor zuigelingen met een medische risico aandoening bepalen (**hoofdstuk 4**). In tegenstelling tot de eerdere onderzoeken, was HRV minder beschermend tegen ernstige rotavirus AGE (vaccin effectiviteit 30%; 95% betrouwbaarheidsinterval (BHI) -36;65%) in deze studie, en er werd geen impact op rotavirus ziekenhuisopnames geobserveerd. Omdat de geobserveerde aantallen laag waren, en een groot deel van de AGE episoden niet op rotavirus waren getest, werd een post-hoc analyse gedaan voor AGE ongeacht oorzaak en rotavirus AGE ongeacht ernst. Dit leverde vergelijkbare en niet significante uitkomsten. Samenvattend suggereren de resultaten dat de werking van rotavirusvaccinatie minimaal is in zuigelingen met een medische risicofactor. De bovengrens van het 95% betrouwbaarheidsinterval was 65% in onze studie. Dat wijkt substantieel af van de gemiddelde waarden van 90% effectiviteit die doorgaans voor gezonde zuigelingen worden gevonden in hoog-inkomen landen, waarbij de ondergrens van de 95% betrouwbaarheidsin¬tervallen 76-84% is. Daarom concludeerden we dat de HRV effectiviteit beduidend lager is in risico kinderen en onderstreept deze bevinding het belang van risicogroep specifiek onderzoek.

Ook is er gekeken naar het voorkomen van bijwerkingen bij kinderen met een medische risico aandoening na rotavirusvaccinatie. We vonden dat ernstige bijwerkingen, geduid als mogelijke vaccinreacties, met een voorkomen van 0.25 per 100 toegediende vaccindoses, vaker optraden dan eerder was beschreven voor de populatie zuigelingen met een medische risico aandoening (**hoofdstuk 5**). De meerderheid van de bijwerkingen waren gastro-intestinaal van aard. Mogelijke causaliteit was gebaseerd op de tijdsrelatie met HRV toediening en biologische plausibiliteit wat betreft de pathofysiologie. Globaal werden er meer (gastro-intestinale) bijwerkingen gemeld voor gevaccineerde premature zuigelingen vergeleken met ongevaccineerden (relatieve risico 1.07, 95% BHI 1.04;1.10). In de kleine groep van 27 zuigelingen geboren na een zwangerschapsduur van minder dan 27 weken, die ondanks hun korte amenorroeduur off-label gevaccineerd waren, werd rotavirusvaccinatie goed verdragen en waren geen veiligheidssignalen. De effectiviteits- en veiligheidsresultaten gezamenlijk beschouwend, adviseren wij dat zorgverleners de risico's en voordelen van rotavirusvaccinatie voor kinderen met een medische risico aandoening steeds per individuele casus afwegen.

Eveneens hebben we onderzocht of rotavirusvaccinatie gunstige niet-specifieke effecten veroorzaakt (**hoofdstuk 6**). Levend-verzwakte vaccins, zoals HRV, worden geassocieerd met positieve niet-specifieke effecten. Dit fenomeen wordt toegeschreven aan getrainde immuniteit, een vaccin geïnduceerde modificatie van het aangeboren immuunsysteem dat het risico op

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en/of ernst van ziekte door een niet-doelwit infectie (i.e. een infectie anders dan waartegen het vaccin beschermd) vermindert. In onze studie was HRV niet beschermend tegen acute ziekenhuisopname wanneer opname voor AGE werd geëxcludeerd (hazard ratio: 0.91 95% BHI 0.76;1.16).Verder vonden we geen reductie in cumulatief aantal opgenomen dagen of incidentie van acute luchtweginfecties, zoals kan worden verwacht op basis van getrainde immuniteit mechanismen. Concluderend vonden wij geen aanwijzingen voor positieve niet-specifieke effecten na rotavirusvaccinatie onder kinderen met een medische risico aandoening.

Tenslotte, de implementatie strategie voor het RIVAR project leverde een vaccinatiegraad van 52% op (95% BHI 51;54%) met een grote variatie in vaccinatiegraad tussen zuigelingen met verschillende medische risicofactoren en tussen ziekenhuizen (**hoofdstuk 7**). In Nederland worden de standaard kindervaccinaties aangeboden via de jeugdgezondheidszorg bij consultatiebureaus. Het stellen van de indicatie voor, en het toedienen van rotavirusvaccinatie gericht op zuigelingen met medische risico aandoening past niet binnen deze infrastructuur en dit vereiste derhalve een andere aanpak. Daarom werd dit gerichte vaccinatieprogramma geïmplementeerd in tweede- en derdelijns pediatrische ziekenhuizen. Gebaseerd op evaluatie enquêtes onder ouders en zorgverleners, en diepte-interviews met betrokken zorgverleners gaven we suggesties voor verbetering van een ziekenhuis-gebaseerd gericht rotavirusvaccinatie programma voor medisch risico zuigelingen. Deze suggesties omvatten een uitgebreid onderwijsprogramma voor betrokken artsen en verpleegkundigen met een toegewijde zorgverlener ter plaatse om inbedding in de reguliere zorg te bewerkstelligen, nationale uitvoeringsrichtlijnen om variatie tussen ziekenhuizen te voorkomen en, afhankelijk van de bestaande infrastructuur en locatie, het betrekken van de jeugdgezondheidszorg voor counseling en vaccintoediening.

De rotavirusvaccinatie (kosten-) effectiviteit, het veiligheidsprofiel voor zuigelingen met een medische risico aandoening en de implementatie van een gericht rotavirusvaccinatie programma werden bestudeerd en geëvalueerd in dit proefschrift. Deze populatie van kinderen met een medische risicofactor zou voorrang moeten krijgen op preventie, aangezien de ziektelast hoog en substantieel is, zelfs in een goed ontwikkeld land als Nederland. Echter, een ziekenhuis-gebaseerd vaccinatie programma exclusief gericht op deze zuigelingen toonde matige bescherming en efficiëntie en is derhalve niet meer kostenbesparend. De werking van vaccins is afhankelijk van gastheer eigenschappen en veranderende epidemiologische condities, welke populatie specifiek onderzoek en continue surveillance van vaccineffectiviteit noodzakelijk maken. De matige bescherming van de huidige orale rotavirusvaccins voor kinderen met een medische risico aandoening benadrukt de noodzaak voor alternatieve en geoptimaliseerde preventieve strategieën voor rotavirus.

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List of publications and conference presentations

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Scientific publications not included in this thesis:

Rouers, E. D. M., Berbers, G. A. M., Dongen, J. A. P. Van, Sanders, E. A. M., & Bruijning-verhagen, P. (2019). Timeliness of immunisations in preterm infants in the Netherlands. ***Vaccine***, 37(39), 5862–5867. https://doi.org/10.1016/j.vaccine.2019.08.006

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Mostert, S., Gunawan, S., Wolters, E., van de Ven, P., Sitaresmi, M., van Dongen, J., Veerman, A., Mantik, M., & Kaspers, G. (2012). Socio-economic status plays important role in childhood cancer treatment outcome in indonesia. ***Asian Pacific Journal of Cancer Prevention***, 13(12). https://doi.org/10.7314/APJCP.2012.13.12.6491

Scientific conferences:

27-05-2021	Presentation (e-poster discussion) on Rotavirus vaccine effectiveness among infants with medical risk conditions in the Netherlands, online at ***European Society of Pediatric Infectious Diseases annual meeting***
26-09-2020	Presentation (e-poster) on Implementing a hospital-based rotavirus vaccination program for medical risk infant: an evaluation, and Individual parent counseling results in high rotavirus vaccination coverage among infants with severe medical conditions, online at ***European Society of Pediatric Infectious Diseases annual meeting***
09-05-2019	Presentation (oral) on Safety and tolerability of rotavirus vaccination among extremely preterm infants in the Netherlands, in Ljubljana, Slovenia, at ***European Society of Pediatric Infectious Diseases annual meeting***

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24-04-2019	Presentation (oral) on Rotavirus incidence and burden of disease among risk-group infants in the Netherlands, in Riga, Latvia, at ***European Expert Meeting on Rotavirus Vaccination***
15-06-2018	Presentation (pitch) on Acute gastro-enteritis: incidentie en ziektelast onder hoog-risico kinderen, in Arnhem, the Netherlands, at ***Nederlandse Vereniging voor Kindergeneeskunde congres***
31-05-2018	Presentation (e-poster discussion) on Rotavirus disease burden among high-risk infants in the Netherlands, in Malmo, Sweden, at ***European Society of Pediatric Infectious Diseases annual meeting***
26-05-2017	Presentation (e-poster) on Rotavirus incidence among high-risk infants in the Netherlands, in Madrid, Spain, at ***European Society of Pediatric Infectious Diseases annual meeting***
22-03-2017	Presentation (poster) on Rotavirus hospitalizations in the absence of rotavirus vaccination, in Utrecht, the Netherlands, at ***European Expert Meeting on Rotavirus Vaccination***
13-01-2012	Presentation on Health-care providers' perspectives on childhood cancer treatment, in Manado, Sulawesi, Indonesia, for all employees of the pediatric hematology-oncology ward of Prof Dr RD Kandou hospital

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"***La dolce far niente***" *– a way of life, which the Italians master.*

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Curriculum vitae

Josephine, call name Fien, van Dongen was born in Amsterdam on September 5th 1989. She grew up in Amsterdam, Addis Ababa (Ethiopia), Cochabamba (Bolivia), Katwijk and Voorburg. She attended secondary education at Gymnasium Haganum in the Hague and graduated in 2007. After a gap year with voluntary work in La Paz (Bolivia) and travelling South America, she studied Medicine at the Vrije Universiteit Amsterdam. As part of the Medicine master she did a research internship at a pediatric hospital in Manado (Indonesia) and travelled South-East Asia. After obtaining her medical degree in 2015, she started working as non-training resident at the pediatrics department of Reinier de Graaf Gasthuis and later Onze Lieve Vrouwe Gasthuis.

She became a PhD candidate in 2016 at the Epidemiology of Infectious Diseases group of prof. dr. Marc Bonten, Julius Center of UMC Utrecht, co-supervised by dr. Patricia Bruijning-Verhagen. Besides her PhD, she completed the postgraduate master in Epidemiology, with a specialization in Infectious Disease epidemiology at Utrecht University. She was also member of the VvE visitation committee that reviews epidemiology curricula of Dutch universities, assisted in teaching epidemiology to medical students and supervised bachelor and master research internships. As of April 2021, she is applying for a doctor in training residency in Pediatrics.

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