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Horizontal transmission of a human rotavirus vaccine strain—A randomized, placebo-controlled study in twins☆

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ABSTRACT

Transmission of excreted vaccine-derived infectious virus from vaccinated to unvaccinated individuals is possible within close contacts. This randomized (1:1), double-blind study evaluated the potential for transmission of human rotavirus vaccine strain, HRV (*Rotarix*™) from vaccine recipients to unvaccinated close contacts (twins). 100 pairs of healthy twins aged 6–14 weeks at the time of Dose 1 of HRV vaccine/placebo were enrolled and one randomly selected twin from each pair received two vaccine doses and the other received placebo doses (at 2 and 4 months of age). Presence of vaccine strain in the stool samples of placebo recipients was an indicator of transmission. Serial stool samples were tested for rotavirus using ELISA at pre-determined time points; rotavirus positive stool samples were tested with RT-PCR and reverse hybridization assay to identify G1P[8] vaccine strain. If G1P[8] vaccine strain was detected, the complete genome was sequenced to assess the similarity between viral isolates. Immunogenicity and safety of HRV vaccine in transmission cases was assessed. 15 transmission cases were reported in 80 evaluable twins who received placebo and the transmission rate was 18.8% (95% CI: 10.9–29.0%). None of the transmission cases was associated with gastroenteritis symptoms. Anti-rotavirus IgA seroconversion was 62.5% (95% CI: 51.0–73.1%) (HRV) and 21.3% (95% CI: 12.9–31.8%) (placebo) 7-weeks post-Dose 2; seroconversion in transmission cases was 26.7% (95% CI: 7.8–55.1%). Genetic variations or amino acid substitutions in transmission cases were similar to that seen in corresponding vaccine recipients. Transmission of HRV vaccine strain to unvaccinated twins living in close contact occurred, however, they were not associated with increased of gastroenteritis. Whether transmission leads to indirect protection among unvaccinated individuals remains unknown at this stage.

1. Introduction

Many earlier studies have demonstrated that rotaviruses, like any other enteric viruses are shed in stools and primarily transmitted through fecal-oral route, person-to-person contact and fomites [1,2]. There has been evidence that rotaviruses may also be transmitted to individuals through respiratory droplets [2–4].

The human rotavirus vaccine strain, HRV mimics natural rotavirus infection, replicates in the intestine of the vaccinated infants and provides protection against future rotavirus infections [5]. Studies with the human rotavirus vaccine have demonstrated that the vaccine virus is shed in the stools of vaccinated infants, with the peak shedding observed on Day 7 after first dose (76–80% of infants after Dose 1 and 18–29% of infants after Dose 2) [6].

Due to the shedding of infectious vaccine virus in stools, there is a theoretical possibility for vaccine virus to be transmitted to unvaccinated or naive infants—a process similar to that observed in natural wild-type rotavirus infection [7]. Such transmissions are possibly expected from any live attenuated vaccines such as oral polio vaccine [8]. The phenomenon of transmission of the rotavirus vaccine strain to unvaccinated individuals raises questions about the safety of the vaccine and the possibility of conferring indirect protection particularly in developing country settings where the vaccine coverage might be incomplete as compared to the developed countries [9]. The current study was the first of its kind that explored the possibility of horizontal transmission of the HRV rotavirus vaccine strain from one twin who received HRV vaccine to the other twin who received placebo living under the same

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household. The immunogenicity and safety of the rotavirus vaccine in transmission cases was also assessed.

2. Materials and methods

2.1. Infants and study design

This phase IIIb, randomized (1:1), placebo-controlled, double-blind study conducted at one urban site in Santo Domingo, Dominican Republic (106260/NCT00396630). Baseline data from all major pediatric hospitals and nurseries was obtained in advance. Parents were informed of the study by presentations at maternity centers, distribution of brochures in health centers and by providing information to pregnant women and new parents visiting maternity centers and vaccination sites. Pairs of healthy twins living in the same household, aged 6–14 weeks at the time of enrolment, born after a gestational period of ≥32 weeks attending local primary healthcare centers, were referred to the site and recruited by the participating physicians.

From each pair of twin, one randomly selected twin received two oral doses of HRV vaccine and the other received two placebo doses at 2 and 4 months of age. Within each pair of twins, Dose 1 and Dose 2 of HRV vaccine/placebo was administered on the same day. In view of providing benefit to the infants receiving placebo during the course of the study, an additional dose of HRV vaccine was administered to all infants (aged < 6 months) at 7-weeks after the second vaccine/placebo dose in an open-labeled manner. All infants received three doses of combined diphtheria, tetanus, acellular pertussis, hepatitis B, inactivated poliovirus and *Haemophilus influenzae* vaccine (DTPa-HBV-IPV-Hib [*Infanrix hexa*™, GSK Biologicals]). Infants were not allowed to take part in the study if they had received any investigational drug or vaccine 30 days preceding the first study vaccine/placebo dose or had a history of allergic disease likely to be exacerbated by the vaccine or had a history of chronic gastrointestinal diseases. They were also excluded if they were immunosuppressed or had an acute disease at the time of study enrolment. Hypersensitivity to the vaccine/placebo and intussusception were adverse events that established absolute contraindication to further administration of vaccine/placebo doses.

This study was conducted between January 2007 and February 2008, following Good Clinical Practice and the Declaration of Helsinki; the protocol and related documents were reviewed and approved by the ethics committee of the study centers. Parents or guardians of the participating twins provided consent for study participation by signing the informed consent form.

2.2. Vaccines

Rotarix™ (HRV) vaccine contained at least $10^{6.0}$ median cell culture infectious dose of the vaccine strain per vaccine dose (1 ml). The placebo had the same constituents as the active vaccine but without the vaccine virus and was identical in appearance to the vaccine. The lyophilized vaccine and placebo were reconstituted with the supplied liquid calcium carbonate buffer before oral administration [10].

2.3. Assessment of transmission

Presence of the vaccine strain in the placebo group for any of the stool samples collected at pre-determined time points was considered a positive transmission case. To evaluate rotavirus antigen shedding (ELISA, Dr. Ward's Lab, USA), stool samples were collected by the parents/guardians in each pair of twins (HRV vaccine/placebo) at pre-determined time points—before the administration of the first and second HRV vaccine/placebo dose (or on the day of vaccination), three times a week (every two days) up to six weeks after each dose of HRV vaccine/placebo and at the post-vaccination blood sampling time point (7 weeks post-Dose 2). To ensure proper stool sample collection, surveillance was performed by a social worker at the time of stool sample collection. The study staff stuck appropriate labels on the stool collection containers to avoid mix-up of samples by the parents/guardians.

Stool samples positive for rotavirus in the vaccine recipients by ELISA suggested the presence of HRV vaccine strain, which in turn indicated viral shedding. If the placebo recipients were found rotavirus positive by ELISA, further confirmation for the presence of HRV vaccine strain was done using the appropriate molecular technique (e.g. Reverse Polymerase Chain Reaction [RT-PCR], sequencing). If an ELISA positive stool sample from placebo recipients for which the vaccine strain is not confirmed, the stool sample was tested for rotavirus G- and P-type using reverse hybridization assay at DDL laboratories, the Netherlands or by any other appropriate molecular technique (e.g. RT-PCR, sequencing) [11].

If rotavirus vaccine strain was detected from the twin receiving placebo, stool samples were further tested to estimate the presence of infectious viral particles (direct culture of stool samples on MA-104 cells for which results were expressed qualitatively). If applicable, full genome of rotavirus was sequenced from twin pairs receiving placebo or the HRV vaccine to evaluate genetic variation.

2.4. Assessment of immunogenicity

At pre-vaccination and 7 weeks post-Dose 2 of HRV vaccine/placebo, serum samples were collected from all the twins for the analysis of anti-rotavirus IgA antibody concentration using ELISA methodology designed by Ward et al. [12,13] at GSK Biologicals Laboratory, Rixensart, Belgium with an assay cut-off of 20 U/ml.

2.5. Assessment of safety

Serious adverse events and all episodes of gastroenteritis (diarrhea [three or more looser than normal stools per day] with or without vomiting) occurring throughout the study period (until 7-weeks after Dose 2 of HRV vaccine/placebo) were recorded by the parents/guardians in the dairy cards. In case of a gastroenteritis episode until 7-weeks after Dose 2, and if the stool sample that is temporally closest to the onset day of the gastroenteritis episode is positive for rotavirus by ELISA, then presence of HRV vaccine strain was evaluated using the appropriate molecular technique (e.g. RT-PCR, sequencing). If the vaccine strain is not confirmed, the stool sample was tested for rotavirus G- and P-type using reverse hybridization assay at DDL laboratories, the Netherlands or by any other appropriate molecular technique (e.g. RT-PCR, sequencing).

2.6. Statistical analyses

A randomization list was generated at GlaxoSmithKline (GSK) Biologicals, Rixensart, using a standard SAS® program. A randomization blocking scheme (1:1 ratio, block size = 2) was used to ensure balance between the treatment arms; a treatment number uniquely identified the vaccine doses to be administered to the same infant.

The study was double-blinded and the parents/guardians of infants, investigator and the study personnel were unaware of the study vaccine administered. No investigator or any person involved in the clinical trial (including laboratory personnel, statisticians and data management) was aware of the treatment groups during the course of the study.

The primary analysis of transmission cases and secondary immunogenicity analysis was performed on the according-to-protocol (ATP) cohort for immunogenicity which included all infants who complied with the protocol, had immunogenicity data

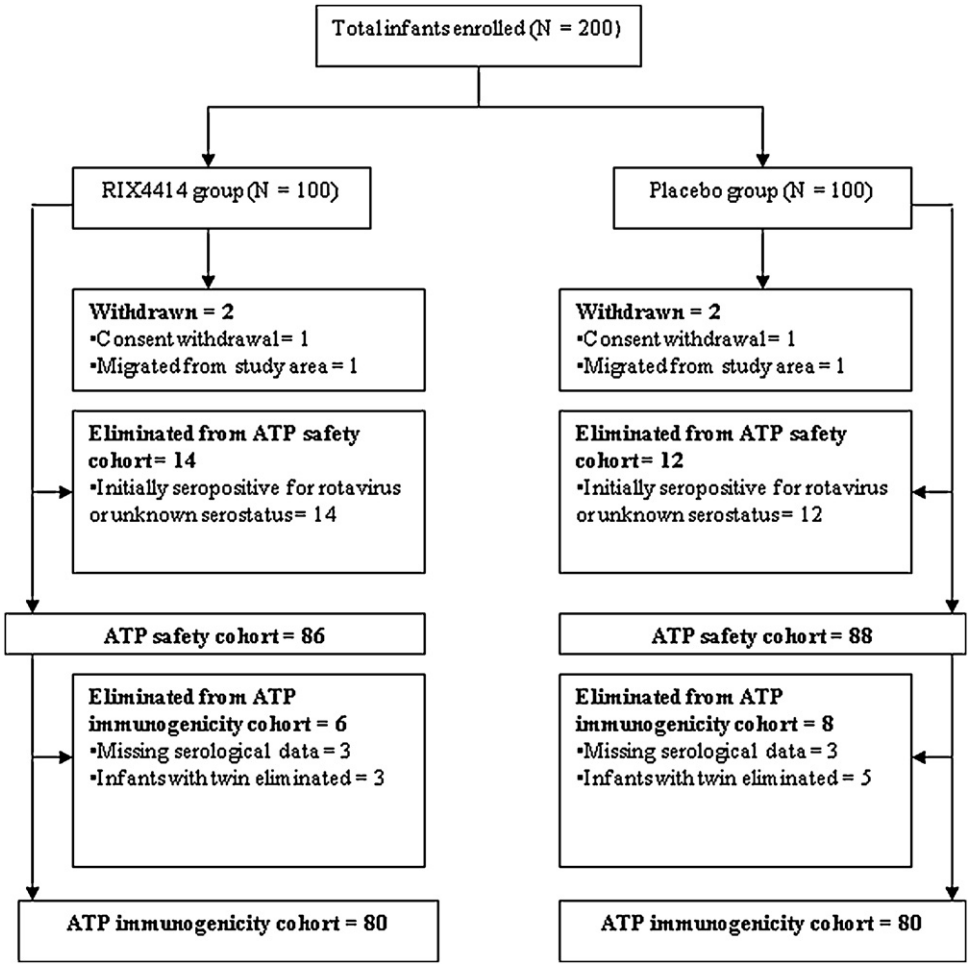

Fig. 1. Distribution of infants.

available at both blood sampling time points, had no rotavirus in stool samples collected at pre-determined time points and whose twin was not eliminated from the ATP immunogenicity cohort. Safety was analyzed on the total vaccinated cohort which included all infants who had received at least one dose of the HRV vaccine/placebo.

The sample size of 200 infants (100 twin pairs) was planned to provide at least 87% power to observe one case of transmission, for a true transmission rate of ≥2%. The percentage of twins receiving placebo with the presence of vaccine strain in at least one stool sample by ELISA was calculated with exact 95% CI [14]. The occurrence of genetic variation in the HRV vaccine strain in the vaccine and placebo recipients was described. As the stool samples were collected three times a week (every two days), the duration of antigen shedding in days was derived as twice the number of rotavirus positive stools and was summarized by group. Live viral load in the twins receiving placebo in the case of transmission was also summarized.

Anti-rotavirus IgA seroconversion rate (anti-rotavirus antibody concentration ≥ 20 U/ml in infants initially negative for rotavirus) and geometric mean concentrations (GMCs) were calculated with their 95% CI [14]. The 95% CI for the mean of log-transformed concentration was first obtained assuming that log-transformed values were normally distributed with unknown variance. The 95% CI for the GMCs were then obtained by exponential-transformation of the 95% CI for the mean of log-transformed titer/concentration.

Gastroenteritis episodes including severe rotavirus gastroenteritis and serious adverse events were tabulated all through the study period.

2.7. Role of funding source

This study was sponsored and funded by GSK Biologicals. The sponsor was involved in all stages of the study, i.e. from study design to data analysis and writing of the report, and also performed rotavirus ELISA testing. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

3. Results

3.1. Demography

One hundred pairs of twins were enrolled to receive at least one dose of HRV vaccine/placebo. Fig. 1 describes the reasons for withdrawal and elimination of infants from the study at each stage. Mean age of the twins at the time of Dose 1 of HRV vaccine/placebo (total vaccinated cohort) was 8.2 weeks (standard deviation: 1.80 weeks). The distribution of male (47.5%) and female (52.5%) infants was similar in the study groups and all infants belonged to the American Hispanic or Latino ethnicity.

Table 1
Summary of the transmission cases.

Transmission case, *N*	Previous dose(s) of vaccine/placebo[a]	Twin receiving HRV		Twin receiving placebo		
		Start day of shedding	Duration of shedding[b]	Day of transmission	Duration of shedding[a]	Live HRV virus detected (yes/no)
1	1	Day 4	8 (D4–D16)	Day 10	8 (D10–16)	Yes
2	1	Day 2	14 (D2–D28)	Day 6	14 (D6–D28)	No
3	2	Day 8	8 (D8–D32)	Day 24	2 (D24)	No
4	1	Day 2	14 (D2–D16)	Day 14	8 (D14–D20)	Yes
5	1	Day 6	16 (D6–D34)	Day 10	4 (D10–12)	No
6	2	Day 10	4 (D10–D12)	Day 12	2 (D12)	No
7	2	Day 2	4 (D2–D4)	Day 8	2 (D8)	No
8	2	Day 2	2 (D2)	Day 8	6 (D8–D12)	No
9	1	Day 2	14 (D2–D18)	Day 14	2 (D14)	No
10	2	Day 4	16 (D4–D36)	Day 30	2 (D30)	No
11	2	Day 10	8 (D10–D16)	Day 8	2 (D8)	No
12	1	Day 10	2 (D10)	Day 8	2 (D8)	No
13	1	Day 8	10 (D8–D16)	Day 4	4 (D4–D6)	Yes
14	1	Day 2	10 (D2–D14)	Day 2	10 (D2–D14)	No
15	2	Day 6	2 (D6)	Day 2	2 (D6)	No

[a] Mean age at Dose 1 was 8.2 ± 1.80 weeks and mean age at Dose 2 was 14.2 ± 1.82 weeks.
[b] Duration of shedding in days was derived as 2 times the number of RV positive stools (the days of the first and last sample positive for RV are presented in brackets). No associated GE symptoms were reported.

3.2. Transmission rate

Of the 80 evaluable placebo-recipient twins, 15 cases of transmission were identified. The percentage of placebo-recipient twins with HRV vaccine strain isolated in at least one stool sample collected at pre-defined time points was 18.8% (95% CI: 10.9–29.0%). Of the 15 transmission cases, eight cases occurred after the first placebo dose and seven cases occurred after the second placebo dose. In 10 transmission cases, HRV vaccine strain was detected in the stool samples of placebo recipients after the twin receiving the HRV vaccine had started excreting rotavirus antigen in the stools. However, in the remaining five transmission cases, HRV vaccine strain was detected in the stool of placebo recipients either before or at the same time of the first detection of rotavirus antigen excretion in the twin receiving the HRV vaccine.

Live virus was identified in three transmission cases and no gastroenteritis symptoms were reported in these infants (Table 1). Samples collected from nine other twins receiving the placebo with presence of vaccine virus antigen in at least one stool sample were tested negative for live virus. The stool samples from three other infants were not tested for presence of live virus due to insufficient quantity of the samples. The mean duration of rotavirus shedding among the transmission cases was 4.7 days in comparison to 8.8 days in the corresponding HRV recipients. None of the 15 transmission cases was associated with any gastroenteritis symptoms.

Most of the rotavirus antigen excretion was observed after Dose 1 of HRV vaccine, with peak excretion observed on Day 6 after Dose 1 (50.0% of infants) and Day 8 after Dose 2 (18.9% of infants). Rotavirus excretion at combined time point was observed in 77.5% (95% CI: 66.8–86.1%) of infants in HRV group.

3.3. Genetic variation

Genetic sequencing of rotavirus genome in the transmission cases (placebo group) and in their respective vaccine-recipient twins revealed that genetic variation was observed mainly in the VP4, VP7, NSP3 and NSP4 genes. The random mutation patterns observed in the transmission cases and their corresponding vaccine recipients were similar. In addition, the transmission cases did not raise any safety concerns with respect to rotavirus vaccine strain reverting to its virulent form or in terms of gastroenteritis episodes.

3.4. Immunogenicity

Anti-rotavirus seroconversion was observed in 50 (62.5% [95% CI: 51.0–73.1%]) HRV recipients and 17 (21.3% [95% CI: 12.9–31.8%]) placebo recipients 7 weeks post-Dose 2. Of the 17 infants who seroconverted in the placebo group, 13 were due to natural infection and four due to vaccine strain transmission (including one of these four infants who presented G1P[8] wild type rotavirus strain in

Table 2
Anti-rotavirus IgA seroconversion rate and GMCs 7-weeks post-Dose 2 of HRV vaccine/placebo (ATP immunogenicity cohort).

	HRV group			Placebo group		
	N[a] (*n*[b])	%[c] (95% CI[d])	GMC[e] value (95% CI)	*N*[a] (*n*[b])	%[c] (95% CI[d])	GMC[e] value (95% CI)
Overall seroconversion rate and GMCs	80 (50)	62.5 (51.0–73.1)	78.6 (50.6–122.2)	80 (17)	21.3 (12.9–31.8)	20.5 (14.5–28.9)
GMCs in seropositive infants	50 (50)	–	271.0 (178.7–411.2)	17 (17)	–	290.6 (129.5–652.4)
Seroconversion rate and GMCs in transmission cases	–	–	–	15 (4[f])	26.7 (7.8–55.1)	248.3 (46.1–1338.4)

[a] *N* = number of infants with available results.
[b] *n* = number of infants with anti-rotavirus IgA concentration ≥ 20 U/ml.
[c] % = percentage of infants with anti-rotavirus IgA concentration ≥ 20 U/ml.
[d] 95% CI = 95% confidence interval.
[e] GMC = geometric mean antibody concentration.
[f] One infant presented the HRV vaccine strain and G1P[8] wild type rotavirus strain in the stool samples.

the stool samples before vaccine strain transmission). The antibody concentrations attained in seropositive infants were 271 U/ml (95% CI: 178.7–411.2) and 290.6 U/ml (95% CI: 129.5–652.4) in the HRV and placebo groups, respectively.

Among the 15 transmission cases, four infants (26.7% [95% CI: 7.8–55.1%]) were seropositive at post-vaccination blood sampling time point (7 weeks post-Dose 2). The anti-rotavirus IgA antibody GMC in these four infants was 248.3 U/ml (95% CI: 46.1–1338.4) (Table 2). The remaining 11 transmission cases had anti-rotavirus GMC < 20 U/ml regardless of virus strain transmission.

3.5. Safety

From Dose 1 to study end, at least one rotavirus gastroenteritis was reported in 10% (95% CI: 4.9–17.6%) of infants in HRV group ($N = 10$) and 6% (95% CI: 2.2–12.6%) of infants in the placebo group ($N = 6$). None of the six rotavirus gastroenteritis stool samples from the placebo recipients contained the HRV G1P[8] vaccine strain whereas in the HRV group, G1P[8] vaccine strain was isolated from one gastroenteritis stool sample. Thus, only one possible case of "vaccine associated" gastroenteritis was observed. Tests to detect pathogens other than rotavirus in the gastroenteritis stool samples were not performed. Therefore, all cause gastroenteritis with G1P[8] vaccine strain shedding was classified as rotavirus gastroenteritis.

SAEs were reported in 11 infants (five in HRV and six in placebo groups), with bronchiolitis and gastroenteritis being the most common SAEs. No fatal SAEs, vaccine-related SAEs or intussusception were reported in this study.

4. Discussion

It is important to study the safety of horizontal transmission of the human live-attenuated rotavirus vaccine virus from the vaccinated infants to the infants who received placebo because of the possibility of conferring indirect protection or the theoretical concern of the ability of these live viruses to mutate and revert to their virulent form. Possible transmission of the HRV vaccine strain to placebo recipients have been observed in earlier clinical trials in infants (5–17 weeks of age at Dose 1) when vaccinated following a 0, 1–2 month schedule. In these studies, HRV vaccine strain was isolated from a total of five placebo recipients and possible transmission may have occurred in the unvaccinated infants [6,15].

In the present study, twins living in the same house were chosen because these conditions were conducive to analyze the true transmission rate between the pairs of twins. A total of 15 cases (18.8%) of transmission were observed in the twins that received placebo based on the detection of HRV vaccine strain antigen from at least one of their stool samples collected. Of these, there were chances that five of the cases were not "true transmission" because in these transmission cases the vaccine virus was isolated from the placebo recipient either before or at the same time as the antigen excreted in the stool samples of the corresponding twin receiving the HRV vaccine (Table 1). The potential explanation for the detection of vaccine virus in the placebo recipients before or at the same time as the vaccine recipients are—firstly, the possible mishandling or contamination of the stool samples, secondly, ELISA test used was not sufficiently sensitive to detect low concentrations of the viral antigen and thirdly, there could have been a short shedding period after vaccine administration (e.g. 1-day, shedding between stool sample collected).

The genetic variations in terms of random mutation patterns observed in the HRV strain isolated from the stools of transmission cases did not result in the rotavirus vaccine stain reverting to its virulent state. None of the transmission cases were associated with gastroenteritis episodes. In addition, significant number of mutations in the transmission cases were observed in the previous clinical studies with the HRV vaccine (unpublished data). These findings confirm that the HRV vaccine strain was stable as demonstrated previously [16].

The phenomenon of transmission has also been observed in studies with other rotavirus vaccines like RRV-TV [7,17]. In a study conducted in Venezuela, horizontal transmission of the RRV-TV vaccine strain to infants receiving placebo was reported in 13% of the total rotavirus gastroenteritis cases. Epidemiological data collected retrospectively in this trial setting revealed that among the unvaccinated population the occurrence of rotavirus diarrhea reduced from 38% to 21% during the vaccination period [7,17]. This supports the concept of indirect protection, where the unvaccinated population appeared to benefit from horizontal transmission.

The peak viral shedding observed in the vaccine recipients was similar to that observed in an earlier Singaporean study [6]. Although shedding of the vaccine virus strain and transmission to the placebo or unvaccinated population questions the safety of the vaccine, the potential benefit of such a phenomenon to the unvaccinated population through the subsequent protective immunity offered is often ignored [7]. Indirect protection is especially critical in poverty-stricken areas of the world where the vaccine coverage rates are low and the unvaccinated population may get protection against rotavirus disease without being actively vaccinated with the rotavirus vaccine.

Immunogenicity results showed that 62.5% (50/80) of infants in the HRV group and 21.3% (17/80) in the placebo group seroconverted for anti-rotavirus antibodies. Four infants (4/15; 26.7%) among transmission cases seroconverted during the study. The remaining 11 transmission cases that did not demonstrate seroconversion had anti-rotavirus GMC < 20 U/ml. In this context, it is important to note that seroconversion alone is not an indicator of protection; however, viral shedding is also an indicator of protection against rotavirus. Earlier efficacy studies with HRV vaccine have consistently shown higher vaccine efficacy against severe rotavirus gastroenteritis even if the seroconversion rate was lower [18,19]. Studies conducted in Singapore [6] and United States [15] identified HRV vaccine strain in the gastroenteritis stools of placebo recipients (two each in Singapore and United States) and anti-rotavirus IgA antibodies in their sera. These findings also indicated the occurrence of possible transmission and subsequent seroconversion in unvaccinated infants.

Similar random mutation patterns in the vaccine and placebo recipients, good immunogenicity and the possibility of indirect protection effect strengthens the position of rotavirus vaccination as the most beneficial strategy in bringing down the worldwide rotavirus disease burden.

The drawbacks of the study are as follows: all stool samples collected were primarily analyzed by ELISA for detection of rotavirus antigen; tests for the detection of other pathogens were not performed. As a result all cause gastroenteritis in infants with shedding was classified as rotavirus gastroenteritis. The ELISA test used for detecting rotavirus shedding in transmission cases may not be sufficiently sensitive to detect low concentrations of the viral antigen.

The results of this study showed that transmission of the *Rotarix*™ (HRV) vaccine strain occurred in twins living in the same household in a developing country. The transmission of the vaccine strain to the placebo recipients was not associated with any safety concerns. Although protection afforded through indirect protection can be expected theoretically, it remains unknown at this stage whether transmission of the HRV vaccine strain to unvaccinated population could indeed help in reducing rotavirus disease burden.

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Rotarix and *Infanrix hexa* are trademarks of GlaxoSmithKline group of companies.

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