Title: Non-secretor histo-blood group antigen phenotype is associated with reduced risk of clinical rotavirus vaccine failure in Malawian infants

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Summary: In case control and cohort studies in Malawi we found little evidence that non-secretor/Lewis negative histo-blood group antigen phenotypes are associated with reduced rotavirus vaccine take in Malawian infants. Non-secretor phenotype was associated with reduced risk of clinical vaccine failure.

Abstract

Background

Histo-blood-group-antigen (HBGA) Lewis/secretor phenotypes are associated with susceptibility to genotype-specific rotavirus gastroenteritis (RVGE). We tested the hypothesis that non-secretor/Lewis negative phenotype leads to reduced vaccine virus replication, IgA response and clinical protection following vaccination with G1P[8] rotavirus vaccine (RV1) in Malawian infants.

Methods

Infants receiving RV1 at age six and ten weeks were recruited to a cohort study. HBGA phenotype was determined by salivary ELISA. RV1 vaccine virus shedding was detected by qRT-PCR in stool collected on alternate days for ten days post-immunization. Plasma rotavirus (RV)-specific IgA was determined by ELISA pre-immunisation and following the second dose. In a case-control study, distribution of HBGA phenotype was compared between RV1-vaccinated infants hospitalized with RVGE and 1:1 age-matched community controls. Rotavirus genotype was determined by RT-PCR.

Results

In 202 cohort participants, neither overall vaccine virus faecal shedding nor seroconversion differed by secretor or Lewis phenotype. In 238 matched case-control infants, non-secretor phenotype was significantly less common in infants with clinical vaccine failure (OR 0.39, 95%CI 0.20-0.75). The prevalence of non-secretor phenotype was less common in infants with P[8] RVGE (OR 0.12, 95%CI 0.03-0.50) and P[4] RVGE (OR 0.17, 95%CI 0.04-0.75). Lewis negative phenotype was more common in infants with P[6] RVGE (OR 3.2, 95%CI 1.4-7.2).

Conclusions

Non-secretor phenotype was associated with reduced risk of rotavirus vaccine failure. There was little evidence of a significant association between HBGA phenotype and vaccine take. These data refute the hypothesis that high prevalence of non-secretor/Lewis negative phenotypes contributes to lower rotavirus vaccine effectiveness in Malawi.

Keywords: rotavirus, HBGA, vaccine, immunogenicity, Malawi

3

Background

Introduction of rotavirus vaccines into childhood immunization programmes has reduced global child deaths from diarrhoeal disease [1], but current vaccines are less effective in low-income, high-mortality countries than in higher income settings [2]. Multiple explanations for this disparity have been proposed, but definitive data are lacking [3]. A widely proposed hypothesis is that histo-blood group antigen (HBGA) phenotype could affect the replication of live rotavirus vaccines in the gut, potentially explaining observed population differences in rotavirus vaccine immunogenicity and effectiveness [4-9].

HBGA are complex carbohydrates expressed on the surface of red blood cells and mucosal epithelial cells. Secretion of HBGA, as free oligosaccharides in saliva and other exocrine secretions, is determined by expression of the FUT2 gene. Mutations of FUT2 result in a non-functional enzyme and "non-secretor" phenotype. A combination of FUT2 and FUT3 gene expression determines the Lewis HBGA phenotype [10].

Rotavirus is a double-stranded RNA virus comprising an eleven-segment genome in a triple-layer protein capsid. Rotaviruses are classified by capsid protein G (glycoprotein VP7) and P (protease-sensitive VP4) genotypes. HBGA glycans have been shown to bind in a strain-specific pattern to the VP8* sub-unit of VP4 [11-15]. In addition, epidemiological studies have shown that HBGA phenotype determines strain-specific susceptibility to RVGE. Secretor and Lewis positive phenotypes have been associated with increased risk of P[8] and P[4] rotavirus gastroenteritis (RVGE) [5, 7, 13, 16-20], and Lewis negative phenotype with increased risk of P[6] RVGE [5, 7].

Both the monovalent human rotavirus vaccine Rotarix® (RV1) and pentavalent human-bovine reassortant vaccine Rotateq®, are based on attenuated P[8] strains. HBGA-associated resistance to P[8] vaccine virus replication could therefore diminish vaccine response. Evidence to support this hypothesis is limited and inconsistent and no data are available from sub-Saharan Africa [5, 6, 8]. Malawi is a low-income country which introduced RV1 nationally in 2012. Malawi has high rotavirus genotypic diversity, with around 20% of RVGE caused by P[6] strains [21]. Rotavirus vaccine effectiveness in the first year of life is estimated at 70% [22]. In this population, we sought to test the hypothesis that intrinsic resistance of Lewis negative/non-secretors to G1P[8] infection results in reduced IgA response, reduced vaccine virus replication and impaired clinical protection against severe rotavirus gastroenteritis following G1P[8] rotavirus vaccine.

Methods

The relationship between HBGA phenotype, vaccine virus replication and rotavirus-specific IgA response was determined in a longitudinal cohort study. The relationship between HBGA phenotype and clinical rotavirus vaccine failure was determined by a cross-sectional case-control study. Ethical approval for both studies was granted by the University of Malawi College of Medicine (P.09/14/1624) and University of Liverpool (00758) Research Ethics Committees.

Study population

Longitudinal cohort study

4

Healthy infants attending a vaccination clinic in Blantyre, Malawi were consecutively recruited, from April 2015 to August 2016, prior to first RV1 immunization, following informed parental consent. Blood samples were taken prior to first RV1 dose (at approximately 6 weeks of age) and two weeks following the second RV1 dose (at approximately 12 weeks of age). Stool samples were taken on days 4, 6, 8 and 10 post immunization.

Case control study

Infants aged between 10 weeks and 1 year with severe gastroenteritis, defined as Vesikari score ≥11 [23], were consecutively recruited, from January 2015 to January 2017, with informed parental consent, from a secondary referral hospital and three primary healthcare centres in Blantyre, Malawi. Stools were tested for rotavirus by rapid immunochromatography test (RotaStrip®, Coris Bioconcept, Belgium). Infants who tested rotavirus positive were recruited as rotavirus gastroenteritis cases (vaccine failures). Age-matched community controls without diarrhoea (for at least one week prior to recruitment), born within ±30 days of rotavirus gastroenteritis cases, were recruited from randomly generated locations within the healthcare catchment areas of each recruitment site in a 1:1 ratio. All cases and controls had received two doses of RV1 vaccine, confirmed by hand-held health records.

Data collection and anthropometry

Socio-economic and demographic data were collected by structured interview. Nutritional status was determined by measurement of length, weight and mid-upper arm circumference (MUAC, a measure of wasting) at time of recruitment, compared to WHO age-determined z scores [24].

Laboratory methods

For detailed laboratory methods see Supplementary Methods. HBGA phenotyping was determined by detection of antigens A, B, H, and Lewis a and b in saliva by ELISA, using specific monoclonal antibodies, detected by peroxidase conjugated anti-mouse IgM. Infants with detectable salivary A, B or H antigen were classified as secretors. Where detection of A, B and H antigens was negative or borderline, secretor status was confirmed by ELISA to detect lectin antigen [25]. Infants who were positive for either Lewis a or Lewis b antigen were classed as Lewis positive, and those negative for both Lewis antigens as Lewis negative. FUT2 genotype was determined for infants of non-secretor phenotype with enough blood available. DNA was extracted from whole blood using the Qiagen DNA Blood Mini Kit (Qiagen, Germany), in accordance with manufacturer's instructions. FUT2 was amplified by PCR and restriction fragment length polymorphism used to identify inactivating mutations.

RV-specific IgA was determined by a custom antibody-sandwich ELISA [26]. Quantification was made by comparison to a standard plasma [27], reported as geometric mean concentration (GMC) in units per millilitre.

Nucleic acid was extracted from stool using the Qiagen Viral RNA Mini-Kit (Qiagen, Germany). Reverse transcription using random primers was used to generate complementary DNA[28]. RV1

5

shedding was determined by vaccine-specific NSP2 real-time polymerase chain reaction (RT-PCR) [29] and confirmed by VP6 quantitative real-time polymerase chain reaction (qRT-PCR) [30] (S1), with a Ct cut-off value for positivity of <40 cycles. In case-control study participants, including community controls, rotavirus infection was defined as VP6 ≥100 copies/ml by qRT-PCR. In both cases and in asymptomatic rotavirus infections in controls, rotavirus genotyping was undertaken using two-stage RT-PCR [31].

Statistical analysis

All statistical analysis was performed in StataIC Version 13.1 (StataCorp, USA).

Cohort Study

RV1 vaccine virus shedding was defined as two or more NSP2 positive, VP6 positive samples post-immunization. NSP2 positive, VP6 negative samples were considered negative. NSP2 negative, VP6 positive samples were assumed to reflect wild-type infection. A minimum of two post-immunization samples were required for inclusion in shedding analysis. Seropositivity was defined as RV-specific IgA >20U/mL. Seroconversion was defined as a change from seronegative pre-immunization to seropositive post-immunization, or at least a four-fold rise in RV-specific IgA concentration post-immunization among infants seropositive at baseline. The relationship between HBGA phenotype (defined categorically on secretor and Lewis status) and these categorical outcomes was assessed by log-binomial regression. The relationship between HBGA phenotype and continuous variables (peak vaccine virus shedding, RV-specific IgA geometric mean concentration) was determined by Wilcoxon rank-sum test.

For the cohort study, a sample size of 200 was estimated to achieve 80% power to detect a risk ratio of 0.5 (versus equal risk, alpha 0.05).

Case-control study

The odds of specific HBGA phenotype (defined categorically on secretor and Lewis status) was compared between cases and matched community controls by conditional logistic regression. With 1:1 controls, a sample size of 123 cases was estimated to achieve 80% power to detect an odds ratio of 2.5 (versus equal odds, alpha 0.05).

Genotyping analysis

In an additional case-control analysis, the distribution of HBGA phenotype by genotype-specific rotavirus gastroenteritis was compared to community controls. This stratified analysis was unmatched, as there were too few matched pairs for meaningful analysis. Separate analyses determined distribution of HBGA phenotype in P[8], P[4] and P[6] rotavirus gastroenteritis compared to community controls by logistic regression. Rotavirus cases where genotype could not be confirmed were excluded.

A descriptive analysis of HBGA phenotype distribution in genotype-specific asymptomatic rotavirus infection in community controls was made.

Results

COHORT STUDY

HBGA phenotype, RV1 faecal shedding and seroconversion

Two-hundred and ninety-three infants were recruited to the cohort study. Of these, 243 infants in the first dose period, 214 infants in the second dose period, and 202 infants in both dose periods, provided at least two stool samples. Both pre- and post-immunisation samples for RV-specific IgA were provided by 196 infants. Demographic characteristics were similar in those with complete data compared to those with incomplete data (Supplementary Tables 1-2).

Compared to secretor infants, non-secretors had significantly reduced risk of vaccine virus faecal shedding in the first dose period, but not in the second. The overall risk of vaccine virus faecal shedding, in infants with data for both dose periods, did not differ between non-secretors and secretors (Table 1).

In a stratified analysis comparing shedding by sampling day, non-secretors had significantly reduced risk of vaccine virus shedding (4/49, 8%) compared to secretors (51/182, 28%) on day 10 following the first vaccine dose. Risk of vaccine virus shedding was not significantly different between non-secretors and secretors on other sampling days in the first dose period, or on any day in the second dose period (Supplementary Table 3). There was no difference in peak level of vaccine virus shedding, as determined by NSP2 cycle threshold (Ct) value by secretor status(Table 1). When Ct values were compared by sample day, median Ct values in non-secretors were higher (viral load lower) compared to secretors on days 6 and 8 following the first vaccine dose, but not on any other sample day (Supplementary Table 4).

There was no difference in vaccine virus faecal shedding between Lewis negative and Lewis positive infants by any categorical or quantitative measure (Table 2, Supplementary Tables 3-4).

Paired serological data were available for 196 cohort infants. Of these infants, 47 (24%) seroconverted. Eleven (6%) infants were seropositive at baseline. The risk of seroconversion was similar in baseline seropositive infants compared to baseline seronegative infants (RR 0.75 (95%CI 0.21-2.7, p=0.66). The risk of seroconversion did not differ by secretor or Lewis phenotype (Tables 1 and 2).

Among infants with detectable post-immunization RV-specific IgA, there was no difference in GMC between secretors and non-secretors, or between Lewis positive and negative infants (Tables 1 and 2).

In a sensitivity analysis where secretor/non-secretor status was re-categorised by confirmatory FUT2 genotyping and phenotype at 10 weeks old, there remained no association between non-secretor status and either vaccine virus shedding or seroconversion (Supplementary Table 5). Concordance between genotype and phenotype was 90%.

There was no difference in vaccine virus shedding or seroconversion when secretor phenotype was stratified by Lewis phenotype (Supplementary Table 6). In a sub-analysis of secretor infants, there was no association between ABO phenotype and either vaccine virus shedding or seroconversion (Supplementary Tables 7-8).

7

CASE CONTROL STUDY

One hundred and nineteen eligible severe rotavirus gastroenteritis cases and 119 age-matched community controls were recruited. Median MUAC was lower in RVGE cases (13.1cm (IQR 12.4-14cm)) than in community controls (13.8cm (IQR 13.2-14.5cm), p<0.01). No other differences in anthropometric or socio-economic characteristics between cases and controls were observed (Supplementary Table 9).

HBGA phenotype distribution in infants with RV1 clinical vaccine failure

The prevalence of non-secretor phenotype was significantly lower in infants with clinical RV1 vaccine failure (14/119, 12%), compared to community controls (33/119, 28%). The odds of non-secretor phenotype were over 60% lower in RV1 vaccine failures than in age-matched community controls (Table 3). In a sensitivity analysis where secretor/non-secretor status was re-categorised by FUT2 genotyping, the distribution of non-secretor phenotype in RV1 vaccine failures and controls was unchanged (OR 0.36, 95%CI 0.17-0.74) (Supplementary Table 10). Concordance between genotype and phenotype was 86%.

There was no association between Lewis phenotype and RV1 vaccine failure (Table 3).

There was no change in observed associations when secretor phenotype was stratified by Lewis phenotype (Supplementary Table 11). In a sub-analysis of secretor infants, there was no association between ABO phenotype and RV1 vaccine failure (Supplementary Tables 12-13).

HBGA phenotype and genotype-specific susceptibility to rotavirus GE

Rotavirus G or P type was confirmed in 116/119 rotavirus gastroenteritis cases. Median virus load in genotyped rotavirus cases was $1.4x10^7$ (IQR $1.5 x10^6$-$4.8x10^7$) copies/ml. P-type was confirmed in 114/119 rotavirus gastroenteritis cases.

Genotype distribution of RVGE cases is shown in Figure 1A. The four most common genotypes accounted for over 75% of genotyped rotavirus gastroenteritis cases: G1P[8] (32%), G2P[4] (26%), G12P[6] (10%) and G2P[6](9%).

The prevalence of non-secretor phenotype was significantly lower in infants with P[8] RVGE (2/47, 4%) and P[4] RVGE (2/38, 5%) compared to community controls (33/119, 28%) (Table 4). All 44 infants with G1P[8] gastroenteritis were secretors. The prevalence of non-secretor phenotype between infants with P[6] RVGE and community controls did not differ (Table 4).

Similarly, the prevalence of Lewis negative phenotype was lower in infants with P[8] RVGE (4/47, 9%) and P[4] RVGE (2/38, 5%) than in community controls (31/119, 26%) (Table 4). In contrast, the prevalence of Lewis negative phenotype was higher in infants with P[6] RVGE (13/33, 39%) than in community controls (Table 4). The odds of infants being Lewis negative were increased over three-fold in those with P[6] RVGE (Table 4) compared to community controls.

HBGA phenotype and asymptomatic rotavirus infection

Asymptomatic rotavirus infection was common: 52/119 (54%) of community controls had detectable rotavirus above 100 copies/ml, with a median viral load of 628 (IQR 258-2008) copies/ml. Due to low viral load, full genotype was only available in 21 asymptomatic infections, and partial genotype in a further 7 (Figure 1B).

The distribution of HBGA phenotypes in genotype-specific asymptomatic infection were similar to those in the wider community control population: 5/16 (31%) infants with P[8] asymptomatic infections and 3/11 (27%) infants with P[4] asymptomatic infection were non-secretors. Three of eight (38%) infants with G1P[8] asymptomatic infection were non-secretors.

Discussion

Contrary to our initial hypothesis, non-secretor phenotype was significantly less prevalent in infants with clinical vaccine failure. We found limited evidence that non-secretor phenotype was associated with reduced vaccine take. The proportion of infants with RV1 vaccine virus shedding in the first dose period was lower in non-secretors compared to secretors, with lower quantitative shedding on some sample days, but the overall risk of vaccine virus shedding, and peak shedding level did not differ. The proportion of infants with post-immunization RV-specific IgA seroconversion was lower in non-secretors compared to secretors but not significantly so. Non-secretor phenotype was associated with protection against both P[8] and P[4] rotavirus gastroenteritis, the two most common rotavirus strains in Malawi. Similarly, against our initial hypothesis, there was no observed association between Lewis negative phenotype and either rotavirus vaccine take or clinical vaccine failure. Lewis negative phenotype was less common in infants with P[8] and P[4] gastroenteritis, but more common in infants with P[6] gastroenteritis, the third most common strain in this study population. These opposing effects may have brought the association between Lewis phenotype and rotavirus vaccine failure toward the null.

The lower point estimate of seroconversion in non-secretor infants (13% compared to 27% in secretor infants) is consistent with previous studies. Bucardo *et al*. (2018) [6] in Nicaragua reported similar findings, while Kazi et al. (2017) [8] in Pakistan reported lower seropositivity following 3 doses of RV1 in non-secretors. Our finding that non-secretor infants are relatively protected from rotavirus gastroenteritis is consistent with data from Bangladesh where non-secretor phenotype was associated with a decreased risk of rotavirus diarrhoea in unvaccinated infants [5]. This study did not demonstrate a significant association between non-secretor phenotype and risk of rotavirus vaccine failure, but numbers of vaccine failures were small. Our findings are also consistent with surveillance data from the US, where non-secretors were at greatly reduced risk of vaccine failure [19], although notably in this population 91% of gastroenteritis cases were due to P[8] infection.

Non-secretor phenotype distribution was similar in infants with asymptomatic rotavirus infection compared to the general study population. This could suggest that non-secretor phenotype provides relative protection against rotavirus disease, but not against asymptomatic infection. This "partial resistance" might explain the limited effect of non-secretor phenotype on vaccine virus shedding. Asymptomatic infection could potentially allow further boosting of protective immunity [32]. Our study is the first to report on the relationship between HBGA phenotype and asymptomatic rotavirus infection. Although the number of infants with asymptomatic infection was high, as observed in other low-income settings[26, 33, 34], the number of genotyped asymptomatic infections was small

9

and conclusions should be considered within this context. However our findings are consistent with data from Lee et al (2018) in Bangladesh, in a prospective cohort including mild diarrhoea, where P[8] infection was not associated with secretor phenotype [5]. Most prior studies on the relationship between HBGA phenotype and rotavirus have focused on hospitalized RVGE. Further data on mild and asymptomatic infections are required to confirm this partial resistance hypothesis.

Our study has several limitations. The lower than expected seroconversion rate may have limited analytic power. Exposure to wild-type rotavirus may have increased post-immunisation seropositivity. However, since non-secretors are protected against wild-type infection, any bias would be toward reduced post-immunisation RV-specific IgA in this group. Subtle differences in vaccine virus shedding may have been underestimated by semi-quantitative measures (Ct value) and borderline results might be clearer in a larger population. Our study relied primarily on salivary HBGA phenotyping by ELISA, which may be less sensitive than genotyping, although concordance between genotyping and phenotyping was high. Furthermore, sensitivity analysis using FUT2 genotyping strengthened the observed protective association between non-secretor type and odds of clinical vaccine failure.

In summary, we found little evidence in this population that non-secretor phenotype was significantly associated with reduced vaccine take. Any possible phenotypic disadvantage in vaccine response was clearly outweighed by non-secretors' relative resistance to wild-type P[8] and P[4] infections, even in this population in which P[6] RVGE was common (>20%). A similar balance would likely exist in other countries with a similar or lower proportion of P[6] RVGE. Recent data show other sub-Saharan African countries have a similar prevalence of P[6] RVGE to Malawi, while the prevalence in all other world regions is substantially lower [35, 36]. While the prevalence of P[6] could vary over time, we contend that HBGA phenotype is highly unlikely to contribute to current population differences in rotavirus vaccine effectiveness between high and low income countries.

11

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14

Table 1: Vaccine virus shedding and RV-specific IgA response by secretor phenotype

	Secretor	Non-secretor	RR (95%CI)	p value
Vaccine virus shedding **1st dose period** n, % (95%CI)	63/188, 34 (27-41%)	10/55, 18 (10-31%)	0.54 (0.3-0.98)	0.04
Vaccine virus shedding **2nd dose period** n, % (95%CI)	58/169, 34 (27-42%)	12/45, 27 (15-42%)	0.78 (0.5-1.3)	0.35
Overall vaccine virus shedding n, % (95%CI)	86/157, 55 (47-62%)	18/45, 40 (26-55%)	0.73 (0.5-1.1)	0.11[a]
Peak vaccine virus shedding[b] **1st dose period** Median Ct (IQR)	29.3 (25.9-32.3)	31.9 (30.4-34.1)		0.13[c]
Peak vaccine virus shedding[b] **2nd dose period** Median Ct (IQR)	32.4 (30.6-34.7)	34.1 (31.9-35.0)		0.21[c]
Seroconversion n, % (95%CI)	41/151, 27 (21-35%)	6/45, 13 (6-27%)	0.50 (0.2-1.1)	0.08[a]
Post-immunization **RV-specific IgA**[d] GMC (95% CI)	109.3 (78.7-151.8)	81.3 (47.9-137.9)		0.52[c]

n=number, %=percent, RR=risk ratio of vaccine virus faecal shedding/seroconversion in non-secretor infants compared to secretor infants. Ct=cycle threshold IQR =inter-quartile range, GMC= geometric mean concentration. a. log-binomial regression b. Peak vaccine virus shedding based on minimum NSP2 RT-PCR Ct value detected within dose period. c. Wilcoxon rank-sum test. d. Only infants with detectable post-immunization RV-specific IgA >20U/ml were included for analysis. This included 24/151(30%, 95%CI 23-38%) secretor and 9/45 (20%, 95%CI 10-35%) non-secretor infants.

Table 2: Vaccine virus shedding and RV-specific IgA response by Lewis phenotype

	Lewis positive	Lewis negative	RR (95%CI)	p value
Vaccine virus shedding **1st dose period** n, % (95%CI)	59/193, 31 (24-37%)	14/50, 28 (17-42%)	0.92 (0.56-1.5)	0.73
Vaccine virus shedding **2nd dose period** n, % (95%CI)	57/169, 34 (27-41%)	13/45, 29 (17-44%)	0.86 (0.52-1.4)	0.55
Overall vaccine virus shedding n, %, RR (95%CI)	84/159, 53 (45-61%)	20/43, 47 (32-62%)	0.88 (0.6-1.3)	0.48[a]
Peak vaccine virus shedding[b] **1st dose period** Median Ct (IQR)	29.8 (26.4-32.4)	31.2 (28.0-34.0)		0.41[c]
Peak vaccine virus shedding[b] **2nd dose period** Median Ct (IQR)	32.1 (30.6-34.7)	33.9 (32.7-35.4)		0.15[c]
Seroconversion n, %, RR (95%CI)	35/149, 24 (17-31%)	12/47, 26 (15-40%)	1.1 (0.6-1.9)	0.77[a]
Post-immunization **RV-specific IgA**[d] GMC (95% CI)	114.5 (84.7-154.9)	74.5 (35.2-157.6)		0.17[c]

n=number, %=percent, RR=risk ratio of vaccine virus faecal shedding/seroconversion in Lewis negative infants compared to Lewis positive infants. Ct=cycle threshold IQR =inter-quartile range, GMC= geometric mean concentration. a. log-binomial regression b. Peak vaccine virus shedding based on minimum NSP2 RT-PCR Ct value detected within dose period. c. Wilcoxon rank-sum test. d. Only infants with detectable post-immunization RV-specific IgA >20U/ml were included for analysis. This included 42/149(28%, 95%CI 21-36%) Lewis positive and 12/47(26%, 95%CI 15-40%) Lewis negative infants.

Table 3:HBGA phenotype distribution in rotavirus vaccine failures and community controls

HBGA phenotype	Prevalence in RV GE cases (Vaccine failures) n, % (95%CI)	Prevalence in Community Controls n, % (95%CI)	Odds ratio[a] (95%CI) p value
Non-secretor	14/119 12 (7-19%)	33/119 28 (20-37%)	0.39 (0.20-0.75) p=0.005
Lewis negative	24/119 20 (14-28%)	31/119 26 (19-35%)	0.70 (0.37-1.3) p=0.27

n=number, %=percent a. Odds ratio of non-secretor/Lewis negative phenotype in vaccine failures compared to age-matched controls, p value determined by conditional logistic regression.

Table 4: HBGA phenotype distribution in genotype-specific RVGE

HBGA phenotype	Community Controls n, %	P[8] RVGE n, % OR[a] (95%CI) p value	P[4] RVGE n, % OR (95%CI) p value	P[6] RVGE n, % OR (95%CI) p value
Non-secretor	33/119, 28	2/47, 4 0.12 (0.03-0.50) 0.004	2/38, 5 0.17 (0.04-0.75) 0.02	7/33, 21 1.1 (0.42-2.7) 0.90
Lewis negative	31/119, 26	4/47, 9 0.26 (0.09-0.80) 0.02	2/38, 5 0.17 (0.04-0.73) 0.02	13/33, 39 3.2 (1.4-7.2) 0.006

n=number, %=percent a. Odds ratio of non-secretor/Lewis negative phenotype in genotype-specific RVGE cases compared to community controls, p value determined by logistic regression.

Figure 1: Common genotypes in RV GE cases and asymptomatic infection

A: Common genotypes in RV GE cases B: Common genotypes in asymptomatic RV infection. Partial genotypes – P or G type only confirmed. Mixed infection – more than one G or P type identified.