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# Investigating factors affecting responses to rotavirus vaccination in a randomised control trial in South Africa

**Fellows, Tamika Daniella**

**2020**
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# INVESTIGATING FACTORS AFFECTING RESPONSES TO ROTAVIRUS VACCINATION IN A RANDOMISED CONTROL TRIAL IN SOUTH AFRICA




**Tamika Daniella Fellows**

Research report submitted in partial fulfilment of the requirements for the Degree MSc Epidemiology and Biostatistics

Supervisor: Dr Michelle Groome, Senior Researcher, Respiratory and Meningeal Pathogens Research Unit

**Johannesburg**

**31st July 2020**

## DECLARATION

I, Tamika Fellows, declare that this Research Report is my own, unaided work. It is being submitted for the Degree of Master of Science Biostatistics and Epidemiology at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.



_____

(Signature of candidate)

31st day of July 2020 in Johannesburg

In memory of my Oupa

Wessel Hendrik Prinsloo

1942 – 2015

and for my Mom and Ouma,

treading softly.

# ABSTRACT

**Introduction:** A randomised, placebo-controlled trial (RCT) investigating the safety and immunogenicity of a parenteral monovalent P2-VP8-P[8] subunit rotavirus (RV) vaccine in South African toddlers and infants showed good immune responses to the vaccine antigen and P[8] rotavirus strains. In addition, there was reduced faecal shedding of Rotarix, an oral replicating vaccine, in vaccinees compared to placebo recipients following a challenge dose of Rotarix, suggesting that the P2-VP8 vaccine may suppress local gut multiplication of RV. This model may have the potential to predict vaccine efficacy of other RV vaccines but requires further investigation. We determined whether serum rotavirus-specific antibody responses correlated with rotavirus faecal shedding.

**Methods:** Rotarix-induced shedding of RV in faecal samples was measured five, seven, and nine days after receiving one dose of Rotarix. Serum immune responses included IgA and IgG directed against P2-VP8-P[8] vaccine antigen (anti-P[8] IgA and IgG) and the whole rotavirus lysate (anti-RV IgA), as well as neutralising antibody (NAb) responses against P[8], P[6], and P[4] RV strains. T-tests or Wilcoxon rank sum tests were used to test whether the mean or median, respectively, of antibody titres differed significantly between dichotomous outcome variables. Pearson chi-squared tests were used to detect associations between categorical variables. Multivariate logistic and linear regression models were derived through manual backward stepwise selection and verified by post-estimation goodness of fit tests.

**Results:** Of 147 infants, 76.9% (n=113) did not shed RV after challenge with Rotarix, whereas 23.13% (n=34) shed RV on at least one day. RV-specific IgA, IgG, and NAb immune responses one month after receiving three doses of P2-VP8-P[8] vaccine or placebo were not significantly associated with RV shedding following Rotarix challenge. However, immune responses tended to be higher in non-shedders than shedders. In contrast, IgA titres (AOR=2.14; 95% CI: [1.53-2.99]; p<0.001) and seroresponse (AOR=1.96; 95% CI: [1.37-2.82]; p<0.001) to P[8] vaccine antigen and IgA titers (AOR=1.96; 95% CI: [1.37-2.82]; p<0.001) and seroresponse (AOR=10.79; 95% CI [2.25-51.85]; p=0.003) to whole RV lysate six-months post-P2-VP8-P[8] were significantly positively associated with RV shedding following Rotarix challenge. Similarly, six-months post-P2-VP8, NAb titers (OR=1.93, 95% CI: [1.34-2.78]; p<0.001) and seroresponse (AOR=4.14; 95% CI: [1.69-15.65]; p=0.004) to RV strain Wa and NAb titers (AOR=2.085; 95% CI: [1.41-3.09];

p<0.001) and seroresponse (AOR= 4.08; 95% CI: [1.36-12.23]; p=0.012) to RV strain 89-12 were also significantly positively associated with RV shedding following Rotarix challenge.

**Discussion and conclusion:** RV faecal shedding following Rotarix challenge is a potential model to assess protection following vaccination, however, our study highlighted the need for additional research before it can be used in the field. Serum RV-specific immune responses measured one-month post-P2-VP8 vaccination tended to be higher in infants that did not shed RV post-challenge than infants who shed. Conversely, RV-specific immune responses measured six months post-vaccination were significantly higher in infants with RV shedding than non-shedders. Immunological endpoints are not sufficient to assess faecal shedding following Rotarix challenge and further studies are needed to assess clinical endpoints in relation to faecal RV shedding, while accounting for potential confounders such as Lewis secretor status and presence of enteric co-infections at vaccination.

# ACKNOWLEDGEMENTS

The primary study on which this this research report is based is as a result of collaboration between PATH Vaccine Solutions, Seattle, Respiratory and Meningeal Pathogens Research Unit, South Africa, National Institute for Communicable Diseases, South Africa, Cincinnati Children's Hospital Medical enter Laboratory for Specialized Clinic Studies, Cincinnati, and the Emmes Corporation. Dr Michelle Groome was also the principal investigator for this study.

I would like to acknowledge my supervisor, Dr Michelle Groome, for her tireless notes, attention to detail, patience, and guidance. I am a better researcher and writer because of you.

I would also like to acknowledge my family down by the beach, and inland, for enduring my absence, keeping me fuelled when I was running on empty, and always sending me the love and support I needed to get this done.

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# NOMENCLATURE

| | |
|---|---|
| AOR | Adjusted odds ratio |
| Ct | Cycle threshold |
| CI | Confidence interval |
| DAG | Directed acyclic graphs |
| dsRNA | Double-stranded ribonucleic acid |
| ELISA | Ezyme-linked immunosorbent assay |
| GMC/T | Geometric mean concentration/titres |
| HBGA | Histo-blood group antigens |
| HIC | High-income countries |
| HIV | Human immune-deficiency virus |
| ID | Identifier |
| IgA | Immunoglobulin A |
| IgG | Immunoglobulin G |
| LIC | Low-income countries |
| mAB | Maternal antibody |
| MH | Maentel-Haenszel |
| mRNA | Mitochondrial RNA |
| NAb | Neutralising antibodies |
| NRRV | Non-replicating rotavirus vaccine, used to refer to the parenterally administered P2-VP8 sub-unit vaccine |
| NSP4 | Non-structural protein 4 |
| OR | Odds ratio |

| | |
|---|---|
| ORV | Oral rotavirus vaccine, used to refer to the live attenuated rotavirus vaccine, Rotarix |
| PCR | Polymerase chain reaction |
| RRV | Replicating rotavirus |
| RV | Rotavirus |
| RCT | Randomized control trial |
| VP | Viral Protein |
| WhlRV | Whole rotavirus lysate |
| WHO | World Health Organization |

# 1 CHAPTER ONE: INTRODUCTION AND LITERATURE REVIEW

## 1.1 BACKGROUND

### 1.1.1 Rotavirus burden

Rotavirus (RV) is a disease which occurs globally and causes severe dehydrating diarrhoeal disease in young children. In 2013 the World Health Organization (WHO) estimated that 215 000 children died as a result of RV infection (World Health Organization, 2016). This corresponds to 37% of all deaths as a result of dehydrating diarrhoea amongst children less than 5 years of age, of which more than 90% occurred in low or middle-income countries (Tate et al., 2016). Almost all children have been infected with RV worldwide by the time they reach 3 – 5 years of age which attests to the very high transmissibility of this virus (World Health Organization, 2013). In two hospitals in Gauteng, South Africa between 2003 – 2005, prior to RV vaccine introduction, it was estimated that RV caused 17 644 – 25 630 hospitalisations annually in children younger than two years of age and between 18.2 and 22.8% of those hospitalized for diarrhoea during this time were identified as RV positive, indicating that it is a significant public health problem (Mapaseka et al., 2010). Treatment of RV involves oral rehydration which can be ineffective in severe cases of gastroenteritis where vomiting is a major concern. In these cases intravenous fluids may be required, but this mode of treatment may not be universally available. As such, primary prevention of RV infection through RV vaccination is necessary to limit adverse outcomes of this disease.

### 1.1.2 Structure and classification of rotavirus strains

RV is a double-stranded ribonucleic acid (dsRNA) virus with a wheel-like structure consisting of three layers, wherein the outer membrane is embedded with spikes (Crawford et al., 2018). The outer capsid consists of immunogenic viral proteins (VP), VP7 and VP4, which are responsible for the outward projections and are proteolytically cleaved to VP8. The middle and inner capsid layers are composed of VP6 and VP2 proteins, respectively (Crawford et al., 2018). Rotavirus strains are classified by their antigenic proteins into groups, subgroups, and serotype. The group (A – G) and subgroup (I or II) are predominantly determined by the composition of the VP6 middle capsid layer. Group A rotavirus strains are responsible for causing most diseases in humans, followed to a lesser extent by Group B and C which can also cause disease in humans. The binary serotype is determined by both VP7 (G-type) and VP4 (P-type), both of these carry epitopes which evoke neutralising antibody responses and which have been used to classify RV strains. There are 14 G-

and 11 P-types, and of those, ten and seven, respectively have been detected in humans (Gray et al., 2008; Hoshino and Kapikian, 2000).

### 1.1.3 Pathogenesis of rotavirus disease

In the human host it has been proposed that VP8 mediates cellular entry into host cells by binding to different glycan receptors such as histo-blood group antigens (HBGAs) (Huang et al., 2012; Liu et al., 2012; Ramani et al., 2013). Other co-receptors such as integrins and heat shock proteins (Guerrero et al., 2000, 2002) have also been proposed. Once the virus has entered into host cells, the capsid layers are removed, viral mitochondrial ribonucleic acid (mRNA) is translated by the host's cellular machinery, and new viral particles are formed. First, double-layered particles are assembled and then bound to the non-structural protein 4 (NSP4) which then transports the virus into the endoplasmic reticulum where the outer capsid layer is assembled (Crawford et al., 2018). Mature virus particles are released from the host cell through vesicular transport mechanisms and cell lysis (Crawford et al., 2018). The subsequent release of NSP4, the viral enterotoxin, from infected cells cause enteroendocrine cells to release hydroxytryptamine (Dong et al., 1997; Zhang et al., 2000) which results in nausea and vomiting through increased regulation of gastrointestinal motility, which in turn triggers increased secretion of sodium chloride and water into intestinal lumen resulting in dehydration and diarrhoea (Hagbom et al., 2011; Istrate et al., 2014). Consequently, RV results in vomiting, fever, and malaise in addition to diarrhoea which can lead to hospitalisation and death due to dehydration in severe cases of gastroenteritis. Transmission of RV occurs through the faecal-oral route through person to person contact as large quantities of RV is 'shed' in stools of infected individuals with diarrhoea (Rivera et al., 2011), while some studies have shown that the virus may also be present in fomites (Butz et al., 1993).

### 1.1.4 Development of rotavirus vaccines

Due to the high burden of diarrhoeal disease globally, the WHO prioritised the development of RV vaccines. A study conducted by Velazquez *et al.* (1996) observed that RV infections conferred immunity to subsequent infections from homotypic, partly- and, fully-heterotypic viral strains and reduced the severity of subsequent infections (Velazquez et al., 1996). These findings bolstered the development of RV vaccines and two safe and efficacious live-attenuated replicating RV vaccines emerged, a pentavalent human-bovine reassortant vaccine, Rotateq®, and a monovalent human attenuated vaccine, Rotarix ®, derived from a wild-type G1P[8] strain. Following the success of double-blind, randomised, placebo-controlled safety and immunogenicity trials in Latin America

(Ruiz-Palacios et al., 2006) and Europe (Vesikari et al., 2007), WHO recommended that these vaccines be included in national immunisation campaigns in the aforementioned nations in 2006 (World Health Organization, 2007), and worldwide in 2009 (World Health Organisation, 2009) following similar successful trials in Africa (Armah et al., 2010; Madhi et al., 2010) and Asia (Zaman et al., 2010). Unfortunately, these vaccines have reduced efficacy and effectiveness in low to middle income countries (LMICs) where burden of diarrhoeal disease is highest compared to high income countries (HICs) (Yen et al., 2014).

### 1.1.5 Efficacy and effectiveness of oral RV vaccines

A systematic review compiled by Leshem *et al.* (2014) illustrated that pooled vaccine effectiveness of Rotarix against homotypic and heterotypic vaccine strains was more than 35% higher in HICs than LMICs (Leshem et al., 2014). The vaccine effectiveness of Rotarix against rotavirus hospitalisation in South Africa was only 57% (95% CI 40-68) in children who received two doses of the oral vaccine, regardless of their HIV status (Groome et al., 2014b). While this translates into a major decrease in the burden of RV disease, these figures are not comparable with findings in HICs. The disparity in vaccine performance was already apparent in rotavirus vaccine efficacy trials, as summarised in Table 1.1 where efficacy was between 85 – 98% in HICs and 40 – 70% in LICs. The problem of reduced efficacy and effectiveness of oral vaccines is not limited to RV vaccines alone but extends to vaccines against other enteric diseases such as poliomyelitis (Patriarca et al., 1991) and cholera (Levine, 2010) which are also transmitted through the faecal-oral route.

### 1.1.6 Possible factors affecting efficacy in LICs

There are several possible factors which have been postulated to be responsible for the modest vaccine performance in LICs but no research to date can solely account for this phenomenon. Many of the hypotheses have focused on a reduction in vaccine titre successfully arriving at the gut, consequently accounting for reduced immunogenicity of live oral RV vaccines. Increased placentally-acquired RV immunoglobulin G (IgG) titres (Moon et al., 2016), increased RV neutralising antibody (NAb) derived activity in breastmilk (Moon et al., 2010; Novak and Svennerholm, 2015), lactoferrin, lactadherin (Moon et al., 2013), or variations in enteric gut microbiome (Velasquez et al., 2017a), all of which have differing distributions between high- and low-income countries, have been postulated as possible factors which could account for insufficient vaccine titres to elicit a response. Several trials have aimed to mitigate this effect by delaying

breastfeeding at the time of immunisation (Groome et al., 2014a), changing vaccination schedules, or increasing the number of doses, all of which had little to no effect on improving vaccine efficacy (Velasquez et al., 2017a). Concurrent administration of oral polio vaccines has been associated with significantly ($p<0.05$) reduced levels of RV-specific IgA seroconversion in South Africa (Steele et al., 2010), Bangladesh, Chile, and Latin America (Velasquez et al., 2017a). However, there is considerable debate as to whether rotavirus-specific IgA seroconversion rates are related to actual vaccine effectiveness (Patel et al., 2013). Another factor which could explain reduced vaccine efficacy is increased susceptibility of the host to RV disease as a result of a genetic predisposition mediated by HBGAs.

**Table 1.1: Results of RV vaccine efficacy trials conducted in multiple different high- and low-income countries**

| Study | SES | Follow-up period | Vaccine | Country | Efficacy | Sample Size |
|---|---|---|---|---|---|---|
| (Phua et al., 2005) | HIC | 18 months | Rotarix | Singapore | 82.0% | 2464 |
| (Ruiz-Palacios et al., 2006) | HIC | 24 months | Rotarix | 11 Latin American countries and Finland | 85.0% (69.6 to 93.5%) | |
| (Vesikari et al., 2007) | HIC | 24 months | Rotarix | Europe (Czech Republic, Finland, France, Germany, Italy, Spain) | 87·1% (95% CI 79·6–92·1) | 3994 |
| (Armah et al., 2010) | LIC | 24 months | Rotateq | Mali | 17·6% (–22·9 to 45·0) | 5468 |
| | | | | Ghana | 55·5% (28·0 to 73·1) | |
| | | | | Kenya | 63·9% (–5·9 to 89·8) | |
| | | | | **Overall** | **39.3% (19.1 - 54.7%)** | |
| (Zaman et al., 2010) | LIC | 24 months | Rotateq | Bangladesh and Vietnam | 48·3% (95% CI 22·3–66·1) | 2036 |
| (Madhi et al., 2010) | LIC | 12 months | Rotarix | Malawi | 49.4 (19.2 - 68.3%) | 4939 |
| | | | | South Africa | 76.9% (56.0 - 88.4%) | |
| | | | | **Overall** | **61.2% (44.0 - 73.2%)** | |

Table notes: Socioeconomic status (SES), low income countries (LIC), high income countries (HIC)

### 1.1.7 Lewis secretor status and immune response

There is an increasing body of evidence to suggest that resistance to infection and disease is mediated by the expression of *HBGA* and the resultant carbohydrates present on the surface of

epithelial cells within the gastrointestinal tract in addition to other systems (Anstee, 2010; de Mattos, 2016; Imberty and Varrot, 2008; Nordgren et al., 2013; Varki and Gagneux, 2012). There are 4 different genes which control the different phenotypes which can occur, namely the *ABO (A, B, AB, or O), H (H or H-deficient)*, *secretor (secretor or non-secretor)*, and *Lewis (positive or negative)* genes. Furthermore, literature suggests that there are population level differences in the distribution of these phenotypes which could predispose some populations towards certain diseases (de Mattos, 2016). This has been demonstrated by Heneghan et al. (1998) where *ABO* and *Lewis HBGAs* influenced *Helicobacter pylori* colonisation and the resultant inflammatory immune response (Heneghan et al., 1998). Nordgren et al. (2013) demonstrated that norovirus infections of children in Burkina Faso were influenced by *HBGA* phenotypes such that only *Lewis-positive* secretors were infected with the P[8] RV strain and not *Lewis-negative* children, whereas the P[6] strain could infect both phenotypes. If there is a high level of *Lewis-negative* children, as observed in Burkina Faso, it is likely that they will be resistant to the P[8] strain which will lead to reduced vaccine infectivity which could explain regional discrepancies in vaccine effectiveness (Nordgren et al., 2014).

### 1.1.8 **Development of alternative RV vaccines**

Due to the low efficacy of currently available RV vaccines in LICs it is necessary to develop alternative RV vaccines to prevent severe and fatal instances of the disease in LIC with the highest burden. Safety and immunogenicity trials of a new non-replicating parenteral vaccine, a P2-VP8 subunit, have recently been completed (Groome et al., 2017a, 2020) and indicate that the vaccine is both safe and immunogenic. Lessons from previous vaccine development indicate that multiple vaccine candidates must be trialled concurrently and rapidly so that the most successful candidate is the best candidate and not the only candidate (Glass et al., 2018). One of the major factors hindering the development of additional vaccines is the lack of reliable correlates of protection for RV. It is not always financially feasible or ethical to assess clinical endpoints during a large randomised efficacy trial where relative incidence of RV infection will be low (Patel et al., 2013; Phua et al., 2005). This is especially true when considering that oral RV vaccines are licensed in many countries and the sample size required to achieve sufficient power for non-inferiority trials is very large. Therefore, correlates of protection are required which may serve as proxy indicators of immune protection against the disease, particularly when trying to examine the effect of small-scale interventions on improving vaccine effectiveness.

### 1.1.9 IgA as a correlate of protection

RV-specific IgA titres have been measured pre and post-vaccination to serve as indicators of immunogenicity in a number of RV vaccine trials (Araujo et al., 2007; Fix et al., 2015; Groome et al., 2017a; Phua et al., 2005). While some studies have shown that increased levels of RV IgA in serum is associated with decreased risk of RV infection and protection against severe infections (Hjelt et al., 1987; Velázquez et al., 2000), other studies conducted by Ward and colleagues (1995, and 1997) showed that increased levels of RV IgA post-vaccination were not significantly correlated with increased levels of protection against RV (Ward et al., 1997; Ward and Bernstein, 1995). A systematic review compiled by Patel *et al.* (2013) indicated that geometric mean concentration (GMC) of IgA titres in excess of 90 are associated with sustained protection against RV infection at a group-level. However, the authors concede that this study is limited in that it has not interrogated the data at an individual level and maintain that RV-specific IgA is still not a complete correlate of protection and needs to be evaluated in the context of the hosts immune response where multiple factors play a role as described in Sections 2.1.5 and 2.1.6 (Patel et al., 2013). One of the major factors contributing to the inconsistent relationship between RV-specific IgA levels and immune protection against live-attenuated oral rotavirus vaccine (ORV) is the location of the immune response. Oral live vaccines are thought to elicit a local immune response within the gastrointestinal tract where the virus is likely to be encountered but this is difficult to measure. Currently, it is more practical to measure serum IgA levels and assume that these levels are correlated with IgA levels within the intestinal tract (Angel et al., 2014).

### 1.1.10 Viral shedding as a correlate of protection

Live oral vaccines are considered advantageous in controlling diseases which are spread through the faecal-oral route as they not only confer direct immunity against disease in the vaccinated, but they also stop the transmission of the disease to unvaccinated contacts. This is why infants are vaccinated with both the non-replicating polio vaccine in addition to the attenuated live oral polio-vaccine. In addition, the live vaccine also results in immunisation of unvaccinated contacts through horizontal transmission of the vaccine strain through viral shedding, a phenomenon which generates a degree of herd immunity within the population (Hsieh et al., 2014a). Upon infection with both naturally occurring RV infections and attenuated live ORVs strains, the viral strains replicate within the gastrointestinal tract and are shed in the stool until the immune system can mount sufficient response to clear the infection (Anderson, 2008; Hsieh et al., 2014a; Yen et al., 2011). Several safety and immunogenicity trials of different ORVs have monitored viral shedding

of the vaccine strain in addition to IgA antibody titres as measures of 'vaccine take', infectivity, and immunogenicity (Araujo et al., 2007; Groome et al., 2017a; Phua et al., 2005).

### 1.1.11 Non-replicating P2-VP8 subunit rotavirus vaccine

Groome et al. (2017) investigated the safety and immunogenicity of a novel parenterally-administered non-replicating RV P2-VP8 sub-unit vaccine in South African infants in a double-blind, randomised, placebo-controlled trial (RCT) (Groome et al., 2017a). In this study, conventional measures of 'vaccine take', such as IgA antibodies to the vaccine antigen and NAbs to rotavirus strains, in addition to a novel method which could be used as a potential proxy for RV vaccine efficacy were assessed. This novel method involves administering an investigational rotavirus vaccine, and then after some time has passed, administering the currently accepted standard of care, which is a live attenuated oral RV vaccine. A live attenuated vaccine is a vaccine that is derived from the original disease-causing pathogen, in this case rotavirus, but has been weakened under laboratory conditions. Therein, the live vaccine form of RV, Rotarix, can still replicate in the host organism (shed) but it will not cause disease. The live attenuated vaccine can then be used to assess participants' immunity to rotavirus by simulating a RV infection or "challenge". The live attenuate vaccine (Rotarix) was administered to participants in placebo and vaccine trial arms one month after the third dose of the parenteral vaccine or placebo was received.

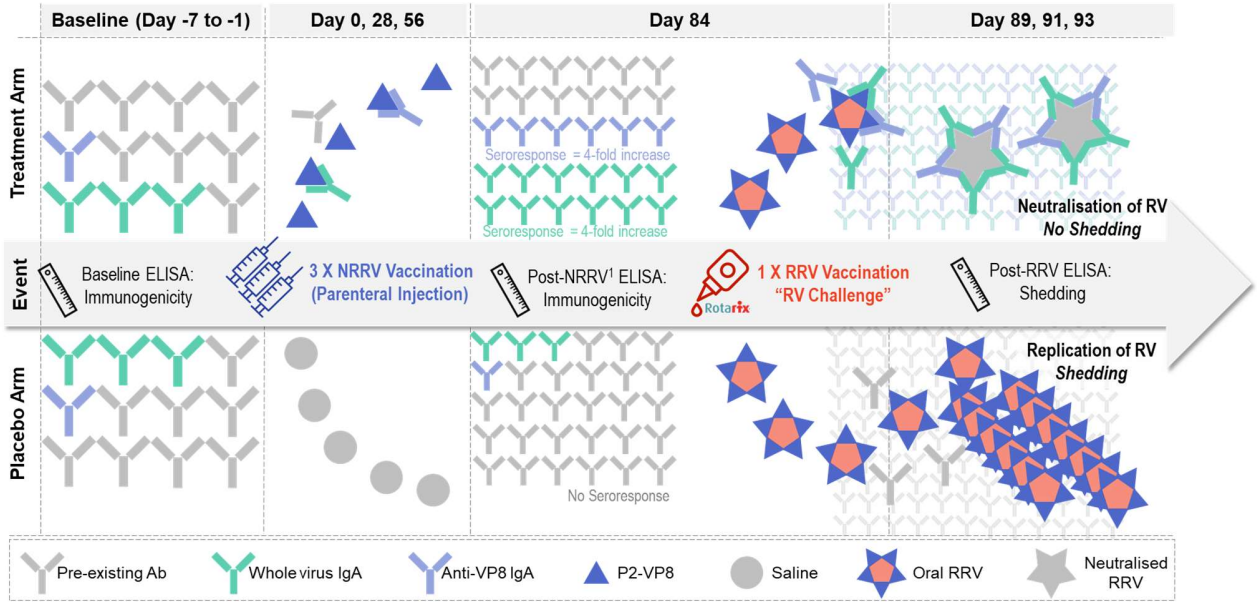



**Figure 1.1: Illustration of the study design employed in a randomised, placebo-controlled safety and immunogenicity trial of the parenterally administered P2-VP8 (NRRV) subunit vaccine (Groome et al., 2017).** Abbreviations: RRV: Replicating rotavirus vaccine; NRRV: non-replicating rotavirus vaccine; ELISA: enzyme-linked immunosorbent assay; Ab: antibodies; IgA: Immunoglobulin A; IgG: Immunoglobulin G; P2-VP8: parenteral P2-VP8 sub-unit vaccine.

Faecal shedding of the oral vaccine strain of Rotarix was measured after it was administered to determine whether infants could suppress local gut multiplication of the oral vaccine strain. While ethically it was required to give children a licenced vaccine (Rotarix ®) after administration of the trial vaccine, there were several advantages to this unique study design. Firstly, it allowed for a unique opportunity to assess vaccine-derived viral shedding as a proxy indicator for immune protection, by assessing whether immunity against RV infection was present when the system was 'challenged' with the live oral RV vaccine strain. Secondly, it showed the ability of the non-replicating rotavirus vaccine (NRRV) to prevent shedding and subsequent horizontal transmission of RV infections. The study found that there was a significant reduction in shedding in the vaccine group compared to the placebo group and concluded that, with additional research, this model has the potential to predict vaccine efficacy of other rotavirus vaccines in the field.

### 1.1.12 **Other strategies for vaccine development**

While sub-unit vaccines like P2-VP8-P[8] have several advantages over live-attenuated oral vaccines, including lack of adverse gastrointestinal reactions in hosts and ease of production (Kardani et al., 2016), they usually only elicit a humoral response while live-attenuated vaccines are capable of eliciting cell-mediated immunity (Kardani et al., 2016). There are several strategies to improve the immunogenicity of sub-unit vaccines, including the use of adjuvants (Lee and Nguyen, 2015; Nascimento and Leite, 2012), which stimulate innate immune responses (Eisenbarth et al., 2008), and prime-boost regimens, which enhance both cellular and humoral immunity (Kardani et al., 2016; Meng et al., 2013; Nascimento and Leite, 2012). Prime-boost regimens involve multiple doses of either the same antigen (homologous) or different antigens (heterologous) at different points in time. This strategy results in greater levels of immunity after each subsequent dose such that individuals who have been primed with an initial dose of the vaccine would illicit greater immune responses after the booster dose (Valdés et al., 2019).

## 1.2 PROBLEM STATEMENT

Due to the lower efficacy of oral RV vaccines in low and middle-income countries like South Africa compared to high-income countries, there is a need to develop new RV vaccines. However, it will be expensive and necessitate large non-inferiority RCTs to licence new rotavirus vaccines using clinical endpoints, consequently, proxy measures of immunity, or correlates of protection, are being investigated in order to assess RV vaccine immunogenicity and efficacy. This would

facilitate licensure of new rotavirus vaccines. Currently there are no absolute correlates of protection for RV which creates a bottleneck in testing of alternative vaccines.

## 1.3 JUSTIFICATION

The study conducted by Groome et al. (2017) was the first study which used suppression of oral RV vaccine shedding as a proxy measure for immune protection following administration of a parenteral vaccine, in addition to currently used measures of immunogenicity. No research to date has investigated the association between shedding of a live-attenuated oral RV virus vaccine strain after vaccination with a parenteral sub-unit RV vaccine, and serum IgA and IgG antibodies to the parenteral vaccine antigen, P[8], IgA antibodies to whole virus lysate for RV, and neutralising antibodies to different RV strains, Wa, Ds1, 1076, and 89-12. There is therefore a need to investigate whether serum rotavirus antibodies (which are induced by the parenteral vaccine) correlate with faecal shedding. Consequently, a secondary data analysis was conducted using the data obtained from the RCT conducted by Groome et al. (2017) which is described above**.**

## 1.4 STUDY OBJECTIVES

### 1.4.1 **Aim**

To investigate whether measured serum immune responses to three doses of a parenteral subunit RV vaccine correlate with stool shedding of vaccine-derived rotavirus in a cohort of healthy infants in South Africa.

### 1.4.2 **Objectives**

1. To assess the concordance of measurement of faecal shedding by enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) testing in infants after receipt of a challenge dose of the ORV, Rotarix.
2. To determine if there is an association between faecal shedding of the live-attenuated oral vaccine strain (Rotarix) following oral challenge and serum RV-specific antibody responses, one and six months after vaccination with the 3$^{rd}$ dose of a parenteral RV subunit vaccine or placebo.
3. To determine whether P2-VP8 IgA antibody responses after P2-VP8 vaccination are affected by baseline serum P2-VP8 IgA titres, IgA titres to the whole RV lysate, and absolute neutrophil count measured in infants vaccinated with three doses of P2-VP8 subunit RV vaccine.

# 2 CHAPTER TWO: APPROACH AND METHODOLOGY

## 2.1 DESCRIPTION OF PRIMARY STUDY

The primary study was a single-centre, dose-escalation, placebo-controlled RCT to investigate the safety and immunogenicity of a new non-replicating parenteral subunit RV vaccine, P2-VP8-P[8] (P2-VP8) in healthy toddlers and infants (Groome et al., 2017a). The clinical trial was conducted at the Respiratory and Meningeal Pathogens Research Unit (RMPRU), with Dr Groome as the principal investigator. The study population consisted of healthy HIV-uninfected South African infants from Soweto whose parents were able to provide written informed consent for their child's participation in the clinical trial and who intended to remain within the vicinity of the study site for the duration of the study. Infants were eligible for the trial if they were between 6 and 8 weeks old, carried to term (gestation period >37 weeks) and determined to be in good health by a clinical and medical history examination. Full details of inclusion/exclusion criteria are described in Appendix A.

The final study sample eligible for the primary analysis is depicted in Appendix B and consisted of 48 infants in the dose-escalation phase of the trial who were enrolled between the 17$^{th}$ of March 2014 and the 29$^{th}$ of September 2014, an additional 114 infants were enrolled between the 3$^{rd}$ of November 2014 and the 20$^{th}$ of March 2015. The final cohort consisted of 162 infants who were allocated to placebo or vaccine in a 1:3 ratio through block randomisation, respectively. Figure 2.1 shows the vaccination and measurement events of the primary study. Infants received three doses of P2-VP8-P[8] vaccine or placebo at approximately 6, 10 and 14 weeks of age. Serum samples were collected from infants at baseline (Day 0: 6 weeks of age) and one and six months after receiving the third dose of the P2-VP8-P[8] vaccine or placebo (Day 84: ~18 weeks of age; and 224: ~9 months of age, respectively). IgA and IgG titres directed against P2-VP8-P[8] vaccine antigen (anti-P[8] IgA and IgG) and the whole rotavirus lysate (anti-RV IgA) were measured, as well as NAb responses against four RV strains: Wa (G1P[8]), 89-12 (G1P[8]), DS-1 (G2P[4]), and 1076 (G2P[6]), as previously described (Ward et al., 1996). Placebo and vaccine trial arms were vaccinated with ORV, Rotarix, which is currently in use in the South African Expanded Program on Immunisation, after they received study vaccine/placebo as protection from the experimental vaccine was not known. This provided a unique opportunity to measure shedding of the Rotarix® strain in stool samples, both qualitatively (ELISA) and quantitatively (PCR), to assess whether vaccine-induced shedding had been suppressed in the vaccine arm relative to the placebo arm.

Three doses of Rotarix were given one month apart. Faecal shedding was measured 5, 7 and 9 days after the first Rotarix dose primarily by ELISA using the ProsPect™ Rotavirus Microplast Assay (Oxoid Ltd, Ely, United Kingdom), according to the manufacturer's instructions. This assay is used for the qualitative detection of Group A rotavirus in faecal samples. The polyclonal antibody used in this assay detects multiple proteins including VP6 which is present in group A rotavirus strains. Infants with a positive ELISA result were considered to be shedders and those with a negative ELISA were considered to be non-shedders. In addition, all stool specimens were subsequently screened using a quantitative VP6-specific reverse transcription (RT)-PCR using primers and probes described by Iturriza Gómara et al., 2002(Iturriza Gómara et al., 2002). Confirmatory genotyping testing was done on ELISA-positive specimens through PCR amplification of the VP7 and VP4 genes to detect G- and P-type RV strains. Specimens which were identified as G1 or P[8] RV strains were sequenced using the BigDye® Terminator v 3.1 cycle sequencing kits (Applied Biosystems) and Genetic Analyzer (Applied Biosystems) to determine if the VP4 and VP7 genes were vaccine-derived or wild-type rotavirus strains. The sequence of events is represented in Figure 2.1.

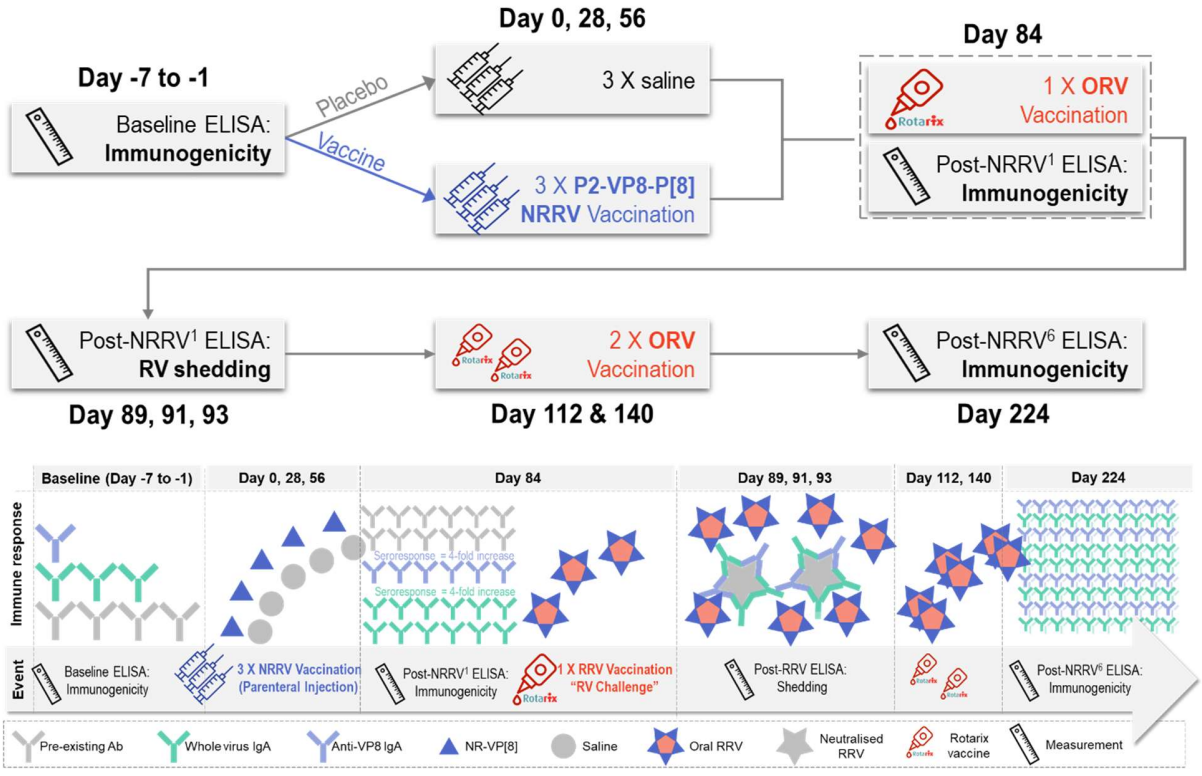



**Figure 2.1: Illustration of events of randomised control trial to assess safety and immunogenicity of P2-VP8-P[8] vaccine, P2-VP8-P[8] conducted by Groome *et al.* (2017).** Abbreviations: RRV: Replicating rotavirus vaccine; NRRV: non-replicating rotavirus vaccine; ELISA: enzyme-linked immunosorbent assay; Ab: antibodies; IgA: Immunoglobulin A; IgG: Immunoglobulin G; P2-VP8: parenteral P2-VP8 sub-unit vaccine.

## 2.2 DESCRIPTION OF SECONDARY STUDY

### 2.2.1 Study design

The primary study was a cohort study with participants who were followed up at various time points. The secondary study was a sub-group analysis of data derived from the primary study to look at how baseline and post-vaccination differences in immune responses are associated with outcome measures one month and six months after receiving three doses of P2-VP8-P[8] vaccine, specifically shedding of RV in stools, following oral Rotarix vaccine administration, and serum RV-specific antibody responses.

### 2.2.2 Study Sample

Only infants were considered in this secondary data analysis. Of 318 infants who consented to be part of the safety and immunogenicity trial, 162 infants were eligible, enrolled and randomised (Figure 2.2). Of these, 45 were assigned to the placebo arm of the trial, while 112 were assigned to receive the P2-VP8 sub-unit vaccine (12 in 10 µg , 47 in 30 µg and 47 in 60 µg groups), and these participants received at least one dose of vaccine. Subsequently, 45 infants in the placebo arm, and 106 infants in the vaccine arm were included in the primary immunogenicity analysis, while 44 infants in the placebo arm, and 103 infants in the vaccine arm were included in the Rotarix shedding analysis. The cohort consisted of placebo and vaccine recipients for the first two objectives, and vaccine recipients only for the third objective.

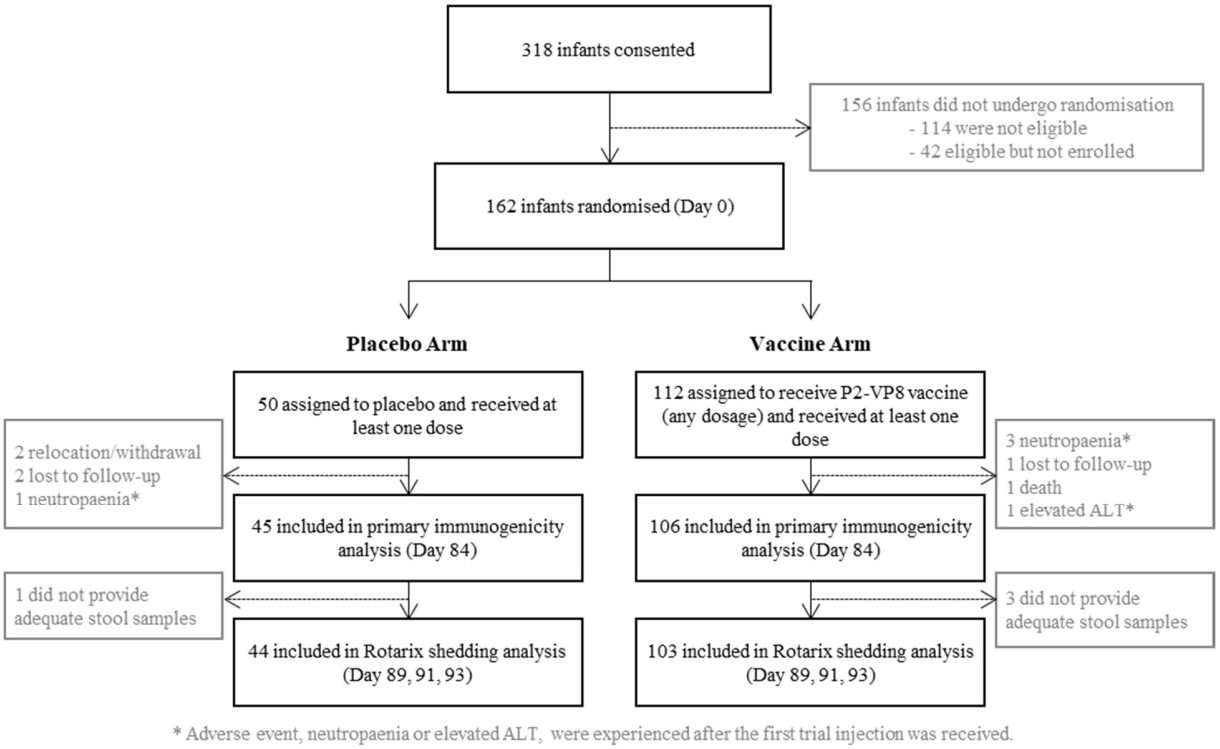



**Figure 2.2: Illustration of study sample included in secondary data analysis from primary study sample.**

### 2.2.3 Power calculation

While sample size was already calculated for the primary study, power was calculated to ensure that there was sufficient power to achieve the second objective. The secondary analysis made use of both continuous and categorical data. The latter was used to calculate the power on the assumption that if power was sufficient for the categorical data it would also be sufficient for continuous data. Individuals from both the placebo and vaccine arms were dichotomously categorised on the basis of their seroconversion and viral shedding status as described in section 4.5. From the primary study, 86 seroconverted (based on anti-P2-VP8-P[8] IgA titres) while 65 did not seroconvert. Assuming that the proportion of individuals who shed the oral vaccine virus was 0.17 and 0.39 in the two groups, respectively (Groome et al., 2017a) then, given a 5% significance level, this analysis had a power of 81.66% to detect a significant difference in shedding between seroconverters and non-seroconverters (WinPepi version 11.65 (Abramson, 2016)).

### 2.2.4 Data collection

All data required to answer the objectives had already been collected during the primary study as described in Section 2.1.

## 2.3 DATA MANAGEMENT

STATA version 15.0 (StataCorp, Texas, USA) was used for all analyses and raw data relevant to the project were de-identified prior to receiving the database. The original raw data from the primary study were checked for missing values and inconsistencies in data quality. This included checks to make sure that all dates, ages, laboratory results, and measurements fell within acceptable limits. The data were received in the form of multiple STATA data files which included a demographics file with a unique participant identifier (ID). There were separate files for each immune response measure which included unique specimen numbers which could be linked to the participant ID in the demographics file. Data were in long format as multiple measurements were collected at multiple time points for each infant. The data were cleaned and converted into wide format and then participant ID and specimen number were used to merge the separate datasets into one complete database.

IgA, IgG, and NAbs were analysed in terms of both the magnitude of the response (continuous variable) and the seroresponse (dichotomous variable). Seroresponse was defined as a four-fold or more increase in the magnitude of the antibody response from baseline to one or six months after receiving three doses of P2-VP8-P[8] vaccine. Infants were categorised as having either a positive or negative seroresponse for each antibody type at each of the one- and six-month time points. Prior to receiving the datasets, post-P2-VP8 IgG and NAb titres were adjusted for the decay of maternal antibodies using the half-life calculated from participants in the placebo group who had detectable baseline titres that were higher than at the post-P2-VP8 visit (Groome et al., 2017a). This adjustment was made by the statistician analysing the data for the primary study. The magnitude of the antibody response was described by geometric mean titres (GMT) unless they were normally distributed and passed tests for equal variance in which case their means and standard deviations were used.

Positively skewed continuous biological variables were described by their geometric means and 95% confidence intervals (CI). Explanatory and predictor variables were only transformed if tests (visual inspection of standardised normal probability plots and skewness and kurtosis tests) for normal distribution of the transformed variable indicated that the distribution was improved. Standardised normal distribution plots were the primary method used for assessment of normality. Variables were defined as having a normal distribution if there were only minor deviations away from normal on the standardised normal probability plot. Wilcoxon Rank-Sum test or Student's T-

test (if the variance was equal) for continuous variables, and Pearson's Chi-Squared tests for categorical variables were conducted to determine if there were differences in the distribution of demographic and immune response factor variables between two groups. P-values less than 5% were considered significant. All variables which were significant at the 10% level were included in the multivariate analyses.

## 2.4 OUTCOMES

### 2.4.1 Concordance of measurement of faecal shedding by ELISA and PCR

As described in Section 2.1, both ELISA and PCR were used to detect faecal shedding of RV. To assess the degree of agreement between the two methods, Mc Nemar's and Cohen's Kappa tests were conducted. The detection limit for RV is approximately $10^2$ cDNA copies/reaction (Hsieh et al., 2014a) which is approximately similar to a cyle threshold (ct) value of 33. Therefore, a ct cut-off value of 33 was initially used to classify PCR samples as positive or negative for RV shedding. Thereafter, Kappa tests were conducted on a range of ct cut-off values between 15 and 36 to identify whether agreement between PCR and ELISA could be improved. Thereafter, Kappa tests were conducted on the range of ct cut-off values to identify whether agreement between PCR and ELISA could be improved. There is some critique of both methods of detection, however, as ELISA has been used most extensively to detect RV shedding in stools and was the primary method of detection in the primary study, this was used as the primary method for this secondary analysis.

### 2.4.2 Association between RV shedding and post-vaccine immune responses

Shedding of RV in stools following Rotarix administration was the primary outcome measure in the second objective which aimed to identify relationships between shedding of RV in stools and serum immune responses in infants one month and six months after receiving three doses of the P2-VP8-P[8] vaccine or placebo. Stool samples were collected five, seven, and nine days after administration of the first dose of Rotarix and tested for the presence of RV as described previously in Section 2.1. The end-point for the primary objective was the proportion of infants shedding RV (determined by ELISA) on at least one day following administration of the first dose of Rotarix.

Serum immune responses measured one month after receiving three doses of P2-VP8-P[8] vaccine were included in this analysis as predictor variables. This included IgA and IgG titres directed against P2-VP8-P[8] vaccine antigen (anti-P[8] IgA and IgG) and rotavirus (anti-RV IgA), as well as NAb responses against four strains of RV, Wa (G1P[8]), 89-12 (G1P[8]), DS-1 (G2P[4]), and

1076 (G2P[6]). Exposure to HIV was determined in the primary study from a number of sources, including history from the mother and use of nevirapine prophylaxis in the infant. Each variable was independently assessed as the main predictor variable through bivariate tests of association using Wilcoxon Rank Sum or Student's t-test (where variance was equal) for continuous variables and Pearson's Chi-Squared or Fishers Exact (where cells values were less than 5) tests for categorical variables. Maentel-Haenszel (MH) tests were used to determine if treatment arm was an effect modifier in the relationship between each predictor variable and RV shedding in stools.

Multivariate logistic regression models were performed using a manual backward stepwise approach and likelihood ratio tests. Each predictor variable was included as the primary variable in a model which was adjusted for treatment allocation (placebo or vaccine), baseline and demographic covariates including age, gender, baseline anti-VP8 IgA and IgG titres, NAb titres to RV strains, and absolute clinical haematology measurements (haemoglobin (g/dL), platelets ($x10^9$/L), neutrophils ($x10^9$/L), lymphocytes ($x10^9$/L), eosinophils ($x10^9$/L), monocytes ($x10^9$/L), and basophils ($x10^9$/L)). The model was constructed by including the main predictor variable of interest and all covariates and then subsequently excluding covariates one by one and running likelihood ratio tests. Variables were removed from the model if the p-value for the likelihood ratio test, where the null hypothesis is that the more parsimonious model is the correct model, was greater than 0.1. The best model was then compared to a model which included an interaction term between the main predictor of interest and treatment allocation and a likelihood ratio test was used to determine if the interaction term improved the fit of the model. The final model was then assessed for confounding by adding in each covariate to determine if the odds ratio for the main predictor variable of interest changed by more than 10%. The final model included the predictor variable of interest, all significant covariates or covariates which modified the odds ratio for the predictor variable by more than 10% and the interaction term, if significant. See Figure 2.3, a theoretical directed acyclic graph (DAG) or causal diagram which was drawn using DAGitty v.3.0 to inform inclusion of variables in the final model.

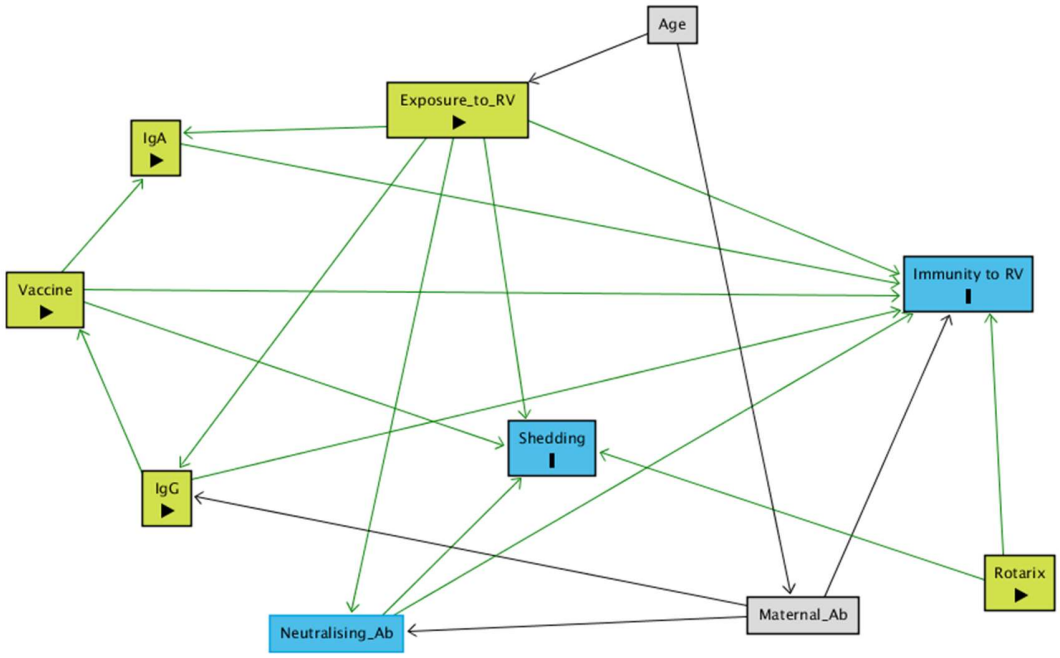



**Figure 2.3: Causal diagram showing the relationship between the immunity to RV, shedding, and the P2-VP8-P[8] vaccine.** Abbreviations: RV: Rotavirus; Ab: antibodies; IgA: Immunoglobulin A; IgG: Immunoglobulin G.

### 2.4.3 Association between post-vaccine IgA immune response to vaccine antigen, P[8], and baseline immune responses

The end-point for the third objective was the magnitude of the anti-VP8-P[8] IgA serum response and the proportion of infants with anti-VP8-P[8] IgA seroresponse one month after receiving three doses of P2-VP8-P[8] vaccine. Anti-P[8] IgA titres were log-transformed to normalise the distribution of the outcome variable. As described in Section 2.4.1, bivariate tests of association were used to detect differences in anti-VP8-P[8] GMTs and the distribution of anti-VP8-P[8] seroresponse by treatment allocation, baseline anti-VP8 IgA and IgG serum responses, clinical haematology and demographic variables. Wilcoxon Rank Sum or t-tests were used to test for differences between continuous variables and Pearson's Chi-Squared tests were used to detect associations between categorical variables. All baseline and immune responses were included in a multivariate linear and logistic regression models with anti-VP8-P[8] titres or anti-VP8-P[8] seroresponse, respectively, as the outcome. A manual backward stepwise approach was used to identify significant covariates ($p>0.1$) as described in Section 2.4.1. Significant interaction terms or confounders which modified the odds ratio by more than 10% were also included in the final model.

# 3 CHAPTER THREE: RESULTS

## 3.1 CONCORDANCE OF MEASUREMENT OF FAECAL SHEDDING BY ELISA AND PCR

According to the ELISA, 76.9% (n=113) of 147 infants did not shed RV at 5, 7 or 9 days after challenge with ORV, whereas 23.13% (n=34) shed RV on at least one of the days. Of 33 shedders whose faecal samples were genotyped, 30 (90.91%) shed RV derived from Rotarix, while 3 (9.09%) shed RV derived from a wild-type rotavirus strain (G9).

### 3.1.1 McNemar's test of agreement

McNemar's test was used to evaluate the difference in agreement between PCR (ct cut-off of 33) and ELISA for the detection of RV in faecal samples, taking the dependent or paired nature of the samples into account. The p-value (<0.0001; 1 degree of freedom) was < 0.05 which indicates that there was a systematic difference between the proportion of positive responses between the two methods (Watson and Petrie, 2010).

### 3.1.2 Cohen's Kappa test

The level of agreement was further investigated using Cohen's Kappa test. There is perfect agreement when kappa is equal to 1; when kappa is equal to 0, the agreement is no better than chance alone. The kappa statistic (0.15: 95% CI -0.01-0.30) was < 0.2, showing there was very little agreement between the two tests when using a ct cut-off of 33 (Watson and Petrie, 2010). This is further indicated by Figure 3.1 where a range of ct cut-off values were investigated to see if there was a cut-off value which would improve the agreement between ELISA and PCR as a detection method. The ct cut-off value that has the best agreement is 21 (Kappa=0.61).

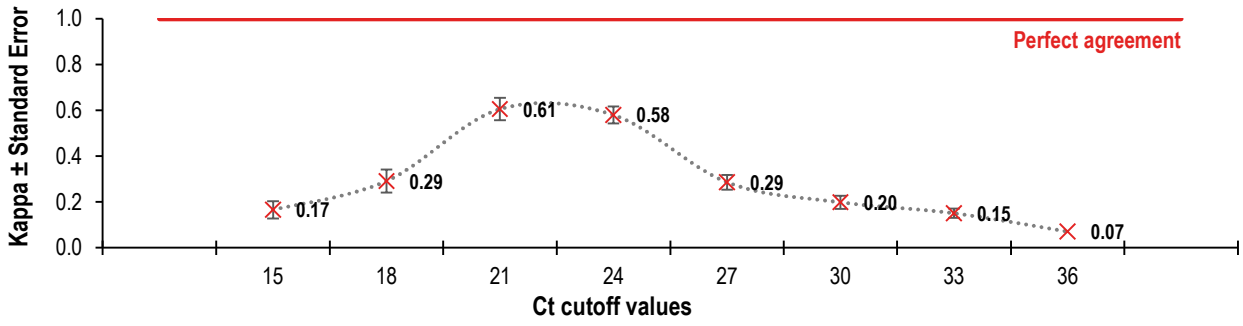



**Figure 3.1: Illustration of Cohen's Kappa statistic comparing PCR and ELISA as detection methods for RV shedding in infant stools for a range of ct cut-off values.**

## 3.2 ASSOCIATION BETWEEN RV SHEDDING (ELISA) AND POST-VACCINE SERUM IMMUNE RESPONSES

### 3.2.1 Factors associated with faecal shedding of rotavirus

Table 3.1 compares the differences in baseline and demographic factors between RV shedders and non-shedders.

**Table 3.1: Differences in baseline demographic and clinical factors between RV shedders and non-shedders, as determined by ELISA, one week after administration of the first dose of Rotarix, in infants receiving three injections of P2-VP8-P[8] vaccine or placebo (N=147).**

| Clinical and demographic factors | RVV Non-shedders (EIA Negative, N=113) | RV Shedders (EIA Positive, N=34) | P-value |
|---|---|---|---|
| **Treatment allocation, n (%)** | | | **0.0040** |
| Placebo | 27 (23.89) | 17 (50.00) | |
| Vaccine | 86 (76.11) | 17 (50.00) | |
| **Treatment allocation, n (%)** | | | **0.0290** |
| Placebo | 27 (23.89) | 17 (50.00) | |
| 10 µg | 10 (8.85) | 2 (5.88) | |
| 30 µg | 39 (34.51) | 6 (17.65) | |
| 60 µg | 37 (32.74) | 9 (26.47) | |
| Sex, n (%) | | | 0.6180 |
| Male | 51 (45.13) | 17 (50.00) | |
| Female | 62 (54.87) | 17 (50.00) | |
| Days of age, mean (95% CI) | 45.64 [45.12-46.16] | 45.29 [44.29-46.3] | 0.5346 |
| Clinical haematology, GM [95% CI] | | | |
| White cell count (x10^9/L) † | 9.79 [9.35-10.25] | 9.31 [8.57-10.12] | 0.2887 |
| Haemoglobin (g/dL) | 11.87 [11.65-12.1] | 11.86 [11.32-12.43] | 0.9963 |
| Platelets (x10^9/L)† | 466.05 [438.8-494.98] | 468.01 [431.17-507.99] | 0.7945 |
| Neutrophil count (x10^9/L)† | 2.16 [2.03-2.3] | 2.1 [1.9-2.32] | 0.4917 |
| Lymphocyte count (x10^9/L)† | 5.99 [5.67-6.32] | 5.57 [5.02-6.19] | 0.2099 |
| Monocyte count (x10^9/L) | 1.09 [1-1.18] | 1.02 [0.84-1.22] | 0.3715 |
| Eosinophil count (x10^9/L) | 0.29 [0.26-0.34] | 0.28 [0.22-0.36] | 0.4566 |
| Basophil count (x10^9/L)† | 0.07 [0.05-0.1] | 0.08 [0.05-0.13] | 0.5806 |
| HIV exposure, n (%) | | | 0.433 |
| Unexposed | 99 (87.61) | 28 (82.35) | |
| Exposed | 14 (12.39) | 6 (17.65) | |

Notes: Wilcoxon Rank Sum was conducted for continuous variables unless variance was equal and a t-test was performed (indicated by a dagger†). Pearson's chi-squared or fisher's exact (indicated by an asterisk*) were conducted for categorical variables. Geometric mean (GM) values were reported for all clinical laboratory results. Abbreviations: GM: geometric mean; HIV: human immunodeficiency virus.

There was an association between treatment allocation and shedding as shown in Table 3.1. Of 34 infants who shed RV in stools, 17 (50.00%) were in the vaccine group compared to 86/113 (76.11%) who did not shed. Shedding in vaccine recipients was significantly reduced compared to the placebo recipients (p=0.004). The unadjusted odds ratio (OR) for the association between shedding and treatment allocation was 0.31 (95% CI 0.13-0.76) which indicates that infants who received the P2-VP8-P[8] vaccine were 69% less likely to be shedders compared to placebo

recipients (p=0.003). Treatment allocation was associated with RV shedding and all subsequent analyses considered treatment allocation.

Neither sex, nor the age of infants at enrolment had a significant effect on RV shedding (p=0.6180 and p=0.5346, respectively). Clinical haematological measurements at baseline were also not associated with RV shedding in infant stools; in all cases, differences between baseline factors and shedding were not significant (p<0.05) at the 5% level as shown in Table 3.1. Exposure to HIV was also not associated with RV shedding (p=0.433). Given the lack of association and limited power to look at this subgroup, this variable was not included as a covariate when assessing other associations.

### 3.2.2 Immune responses to P2-VP8-P[8] vaccination

Immune responses, one- and six-months after receiving three doses of P2-VP8-P[8] vaccine or placebo, were analysed to determine if they were associated with RV shedding in faecal samples following Rotarix vaccine challenge.

### 3.2.3 IgA serum immune responses

The bivariate analysis, which tested for an association between RV shedding and IgA antibodies to the P2-VP8-P[8] sub-unit vaccine antigen and whole rotavirus lysate is shown in Table 3.2.

**Table 3.2: Magnitude and seroresponse rate of IgA antibody responses to P2-VP8 sub-unit vaccine antigen measured at baseline and one and six months after receiving three doses of P2-VP8 sub-unit vaccine or placebo (post-P2-VP8), between RV shedders and non-shedders, as determined by ELISA (N=147).**

| IgA antibody response | RV Shedders (ELISA Positive N=34) | RV Non-shedders (ELISA Negative, N=113) | P-value |
|---|---|---|---|
| **IgA to P[8]** | | | |
| Baseline, GMT [95% CI] | 6.39 [4.84-8.43] | 5.95 [5.21-6.81] | 0.674 |
| One-month post-P2-VP8 magnitude, GMT [95% CI] | 23.11 [14.35-37.21] | 33.76 [25.94-43.96] | 0.240 |
| One-month post-P2-VP8 seroresponse rate, n (col%) | | | 0.222 |
| Negative | 18 (52.94) | 46 (41.07) | |
| Positive | 16 (47.06) | 66 (58.93) | |
| **Six-months post-P2-VP8 magnitude, GMT [95% CI]** | **241.98 [130.62-448.29]** | **55.48 [42.42-72.55]** | **<0.001** |
| **Six-months post-P2-VP8 seroresponse rate, n (col %)** | | | **0.054** |
| Negative | 4 (12.12) | 31 (28.70) | |
| Positive | 29 (87.88) | 77 (71.30) | |
| **IgA to whole RV lysate** | | | |
| Baseline, GMT [95% CI] | 8.41 [7.20-9.83] | 9.73 [8.51-11.14] | 0.264 |
| **One-month post-P2-VP8 magnitude, GMT [95% CI]** | **7.79[7.35-8.24]** | **12.36 [9.78-15.62]** | **0.041** |
| **One-month post-P2-VP8 seroresponse rate, n(col %)** | | | **0.064** |
| Negative | 32 (100.00) | 100 (90.09) | |
| Positive | 0 (00.00) | 11 (9.91) | |
| **Six-months post-P2-VP8 magnitude, GMT [95% CI]** | **229.82 [142.15-371.55]** | **81.44[62.36-106.36]** | **0.001** |
| **Six months post-P2-VP8 seroresponse rate, n (col %)** | | | **0.014** |
| Negative | 3 (9.68) | 34(31.78) | |

| IgA antibody response | RV Shedders (ELISA Positive N=34) | RV Non-shedders (ELISA Negative, N=113) | P-value |
|---|---|---|---|
| Positive | 28 (90.32) | 73 (68.22) | |

Table notes: Wilcoxon Rank Sum was conducted for continuous variables unless variance was equal and a t-test was performed (indicated by a dagger†). Pearson's chi-squared or fisher's exact (indicated by an asterisk*) were conducted for categorical variables. IgA to P[8] refers to the IgA antibody response to the P2-VP8 vaccine antigen; one-month post-P2-VP8 refers to the analysis of sera conducted one-month after receiving three doses of parenteral P2-VP8-P[8] vaccine or placebo; six-months post-P2-VP8 refers to the analysis of sera conducted six months after receiving three doses of parenteral P2-VP8-P[8] vaccine or placebo; mAb adjusted refers to titres which have been adjusted for the decay of maternal IgG antibodies in infants; seroresponse is a categorical variable which is positive if the magnitude of the IgG antibody response to P2-VP8 vaccine antigen measured either one- or six-months after receiving three doses of P2-VP8 vaccine or placebo is four-fold greater than the same response measured at baseline. Abbreviations: RV: rotavirus; IgA: immunoglobulin A; GMT: geometric mean titres; n: number of observations; col%: column percentages; RV: rotavirus; CI: confidence interval; ELISA: enzyme-linked immunosorbent assay.

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The differences in the magnitude of the IgA immune response between RV shedders and non-shedders is depicted graphically in Figure 3.2.

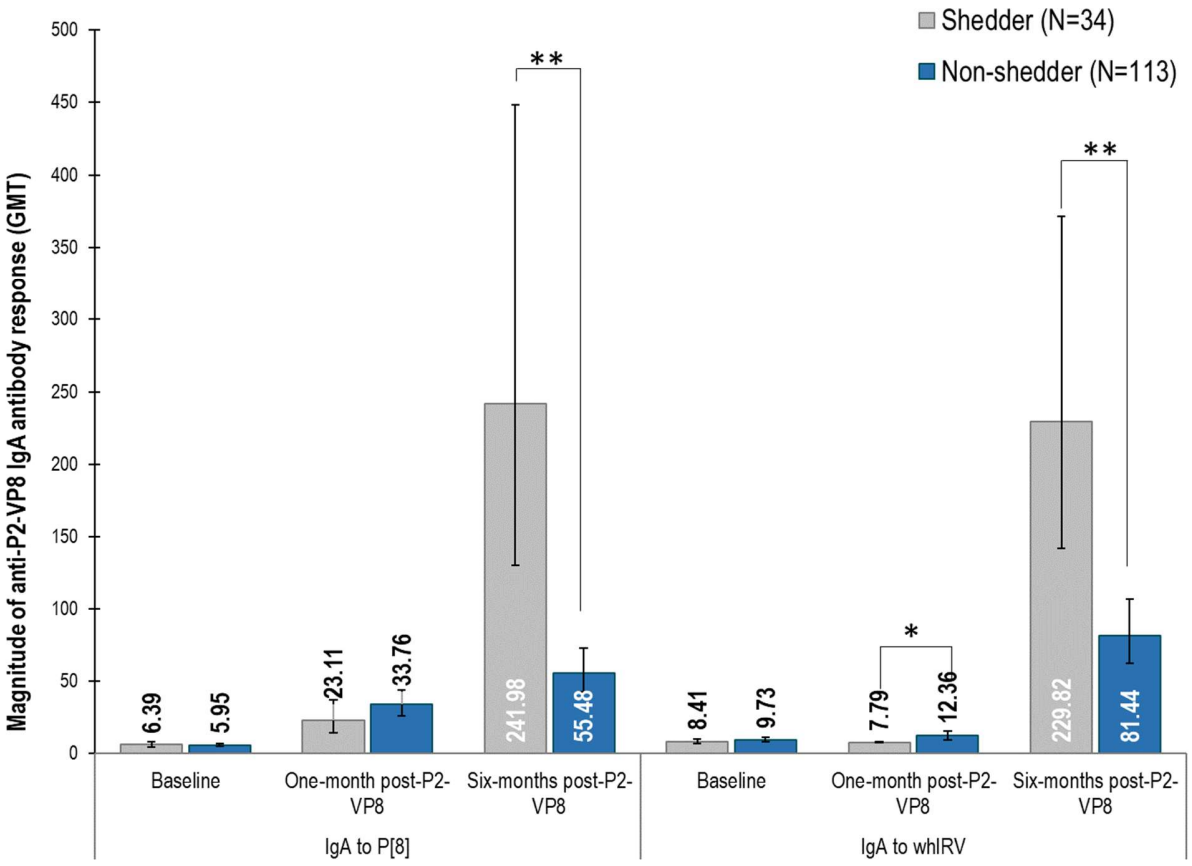



**Figure 3.2: Difference in magnitude of IgA antibody response (GMT ± 95% CI) to P2-VP8 sub-unit vaccine antigen or whole rotavirus lysate between infants who shed RV (shedder) and infants who do not shed RV (non-shedder), as determined by ELISA (N=147).** IgA antibody response was measured at baseline and one and six months after receiving three doses of P2-VP8 sub-unit vaccine (post-P2-VP8). Abbreviations: IgA to P[8]: antibody response to P2-VP8 sub-unit vaccine antigen; IgA to whlRV: antibody response to whole rotavirus lysate; GMT: geometric mean titres. Asterisk indicates significance at the 5% level* or at the 1% level**.

#### *3.2.3.1 IgA Antibodies to P[8] vaccine antigen*

*a. Magnitude one-month post-P2-VP8 vaccination*

The difference in the magnitude of IgA antibodies to P[8] did not differ significantly between shedders (6.39 GMT; 95% CI 4.84-8.43) and non-shedders (5.95 GMT; 95% CI 5.21-6.81) at baseline (p=0.647) (Table 3.2). As shown in Figure 3.2, the magnitude of the IgA response to P[8] is much larger one month after receiving three doses of P2-VP8-P[8] vaccine or placebo compared to baseline. The difference (10.7 titres) between shedders (23.11 GMT; 95% CI, 14.35-37.21) and non-shedders (33.76 GMT; 95% CI 25.94-43.96), although not significant (p=0.240), is also much larger than the difference at baseline (0.4 titres). The difference occurs in the expected direction, with infants who did not shed RV having a higher immune response than infants who did shed RV. The MH test confirmed that treatment allocation was not an effect modifier in this relationship, one-month post-P2-VP8-P[8] vaccine or placebo, as there was insufficient evidence to reject the null hypothesis that the stratified odds ratios for placebo and vaccine recipients were equal (p=0.359). Unadjusted logistic regression confirmed that there was no association between one-month post-P2-VP8-P[8] vaccine IgA antibodies to P[8] and shedding as the p-value for the Wald test was not significant (OR: 1.00: p=0.376) which indicates that there is insufficient evidence to reject the null hypothesis that the coefficient is different from zero at the 5% level of significance.

Table 3.3 shows the final adjusted logistic regression model, derived from backward selection, displaying the relationship between log-transformed IgA antibody titres to P[8] one-month after receiving the third injection and RV shedding. Only treatment allocation was found to be a significant covariate. No other confounders which modified the OR by more than 10% were identified. The adjusted odds ratio (AOR) indicates that for every 2.72[1]-fold increase in antibody titres to P[8], one-month post-P2-VP8 vaccination or placebo, there was no increase in the odds of shedding RV in stools after Rotarix administration (AOR=0.98; 95% CI [0.72-1.35]). Therefore, there is no association between the magnitude of the IgA antibody response to P[8] post-P2-VP8 vaccination at the one-month time point and RV shedding in infant stools, after adjusting for treatment allocation.

[1] Natural log was used in transformation which means base is equal to $e$ *(2.72)*.

**Table 3.3: Final logistic regression model showing the relationship between the magnitude of the IgA antibody response to P2-VP8 sub-unit vaccine antigen P[8], one-month post-P2-VP8, and shedding of RV, as determined by ELISA (n=147).**

| Variables | AOR | z | P>z | 95% CI |
|---|---|---|---|---|
| IgA to P[8] one-month post-P2-VP8 (log titres) | 0.98 | -0.11 | 0.909 | [0.72-1.35] |
| Treatment allocation | | | | |
| Vaccine* | 0.32 | -2.46 | 0.014 | [0.13-0.80] |
| *Constant* | *0.66* | *-0.82* | *0.410* | *[0.24-1.78]* |

Table notes: IgA to P[8] refers to the magnitude of the IgA antibody response to the P2-VP8 vaccine antigen; one-month post-P2-VP8 refers to the analysis of sera conducted one month after receiving three doses of parenteral P2-VP8-P[8] vaccine or placebo.

*b. Magnitude six-months post-P2-VP8*

In comparison, six months after receiving three doses of P2-VP8-P[8] vaccine or placebo, the magnitude of IgA antibody titres to P[8] was much larger than that at baseline or one-month time points. Shedders (241.98 GMT; 95% CI [130.62-448.29]) had a significantly higher IgA immune response to P[8] than non-shedders (55.48 GMT; 95% CI [42.42-72.55]; (p<0.001)).

Unadjusted logistic regression indicated that there was an association between log-transformed post-P2-VP8-P[8] vaccine IgA to P[8] at the six month time point and RV shedding in stools (OR: 1.84, 95% CI: [1.38-2.44]; p<0.001).The odds ratio indicates that for every 2.72-fold[2] increase in IgA antibody titres to P[8], there is a 1.84-fold increase in the odds of having shed RV in stools after Rotarix vaccination. The MH test indicated that treatment allocation was not an effect modified (p=0.790).

**Table 3.4** shows the final adjusted logistic regression model, derived from backward selection, displaying the relationship between IgA antibody titres to P[8] at the six-month time point and RV shedding. IgA antibody titres at baseline and the one-month time points were found to be significant covariates. No other confounders which modified the OR by more than 10% were identified. The likelihood ratio test indicated that including an interaction term to account for the effect of treatment allocation on the relationship between IgA antibody titres to P[8] and RV shedding was not required and the more parsimonious model was favoured (p=0.4020). The AOR indicates that for every 2.72-fold increase in antibody titres to P[8] six-months post-P2-VP8-P[8] vaccine or placebo, there is a 2.14-fold increase in the odds of having shed RV in stools after Rotarix administration (AOR=2.14; 95% CI: [1.53-2.99]; p<0.001), after adjusting for IgA-P[8] at baseline and one month post P2-VP8 vaccination. This relationship is significant. Post-estimation tests

[2] Natural log was used in transformation which means base is equal to $e$ *(2.72)*.

indicated that this model had been correctly specified. Similar backward selection processes were conducted with the log-transformed titres; however, the same model was derived.

**Table 3.4: Final logistic regression model showing the relationship between the magnitude of the IgA antibody response to P2-VP8 sub-unit vaccine antigen P[8] six months post-P2-VP8 vaccination and shedding of RV, as determined by ELISA (n=138).**

| Variables | AOR | Z | P>z | 95% CI |
|---|---|---|---|---|
| IgA to P[8] six-months post-P2-VP8 (log) | 2.14 | 4.48 | <0.001 | [1.53-2.99] |
| IgA to P[8] one-month post-P2-VP8 | 0.99 | -2.37 | 0.018 | [0.98-0.99] |
| IgA to P[8] at baseline | 1.02 | 1.49 | 0.136 | [0.99-1.05] |
| *Constant* | *0.01* | *-5/33* | *<0.001* | [0.02-0.06] |

Table notes: IgA to P[8] refers to the magnitude of the IgA antibody response to the P2-VP8 vaccine antigen; one-month post-P2-VP8 refers to the analysis of sera conducted one month after receiving three doses of parenteral P2-VP8-P[8] vaccine or placebo; six-months post-P2-VP8 refers to the analysis of sera conducted six months after receiving three doses of parenteral P2-VP8-P[8] vaccine or placebo. Baseline refers to the analysis of sera conducted at baseline (enrolment). AOR: Adjusted odds ratio; CI: confidence interval.

### *c. Seroresponse rate one-month post-P2-VP8*

Of 34 who did not shed, IgA serum anti-P2-VP8-P[8] seroresponse one month after the third injection was shown in 16 (47.06%), compared with 66/112 (58.93%) who did not shed. IgA serum anti-P2-VP8-P[8] seroresponse was slightly higher in those who did not shed but this difference was not statistically significant (p=0.222).

The MH test indicated that treatment allocation was an effect modifier in this relationship, the p-value for the homogeneity of ORs indicated that there was sufficient evidence to reject the null hypothesis that the ORs stratified by treatment allocation were equal (p=0.012) and the OR for placebo and vaccine should be reported separately. Although the ORs were only marginally significant for either vaccine or placebo recipients, it is worth noting that for vaccine recipients, RV shedding was higher amongst those with positive anti-P[8] IgA seroresponse compared to those with negative seroresponse (OR=3.49; 95% CI: [0.72-16.84]; p=0.097). Whereas for placebo recipients, RV shedding was lower amongst those with positive seroresponse than those with negative seroresponse (OR=0.15; 95% CI: [0.01-1.49]; p=0.060).

**Table** 3.5 shows the final adjusted logistic regression model, derived from backward selection, displaying the relationship between the IgA seroresponse rate to P[8] and RV shedding. According to this model, IgA seroresponse rate to P[8] was not significant (p=0.089) at the 5% level. Treatment allocation was the only significant covariate (p=0.003). Both IgA antibodies to whole RV lysate and IgG antibodies to P[8] at baseline were included in the model as confounders which modified the OR for IgA to P[8] seroresponse by more than 10%. The likelihood ratio test for a model which included an interaction term between the IgA seroresponse rate to P[8] and treatment allocation indicated that including the interaction term improved the fit of the model (p=0.012).

There was no significant association between RV shedding and serum IgA seroresponse to P[8], after adjusting for treatment allocation and baseline serum IgA to whole viral lysate, IgG to P[8] and NAb to Wa.

**Table 3.5: Final logistic regression model showing the relationship between the anti-P[8] IgA seroresponse one-month after the third injection and RV shedding, as determined by ELISA (n=143).**

| Variables | AOR | Std. Err. | z | P>z | 95% CI |
|---|---|---|---|---|---|
| IgA to P[8] one-month post-P2-VP8 seroresponse | | | | | |
| Positive | 0.15 | 0.17 | -1.70 | 0.089 | [0.02-1.34] |
| Treatment allocation | | | | | |
| Vaccine | 0.09 | 0.07 | -2.99 | 0.003 | [0.02-0.43] |
| Treatment allocation#IgA to P[8] one-month post-P2-VP8 seroresponse | | | | | |
| Vaccine#Positive | 22.59 | 31.18 | 2.26 | 0.024 | [1.51-337.92] |
| IgA to whole RV lysate at baseline | 0.99 | 0.01 | -0.79 | 0.429 | [0.96-1.02] |
| IgG to P[8] at baseline | 1.00 | 0.00 | -0.28 | 0.783 | [1.00-1.00] |
| NAb to Wa at baseline | 1.00 | 0.00 | -0.52 | 0.602 | [1.00-1.00] |
| *Constant* | 1.18 | 0.50 | 0.38 | 0.700 | [0.51-2.71] |

Table notes: IgA to P[8] seroresponse is a categorical variable which is positive if the magnitude of the IgA antibody response to the P2-VP8 vaccine antigen measured one-month after receiving three doses of P2-VP8 vaccine or placebo is four-fold greater than the same response measured at baseline. Baseline refers to the analysis of sera conducted at baseline (enrolment); one-month post-P2-VP8 refers to the analysis of sera conducted one month after receiving three doses of parenteral P2-VP8-P[8] vaccine or placebo; six-months post-P2-VP8 refers to the analysis of sera conducted six months after receiving three doses of parenteral P2-VP8-P[8] vaccine or placebo; IgG to P[8] refers to the magnitude of the IgG antibody response to the P2-VP8 sub-unit vaccine antigen at baseline. NAb to Wa refers to the neutralising antibody response to rotavirus strain Wa at baseline; AOR: adjusted odds ratio; Std. Err: standard error; CI: confidence interval.

### *d. Seroresponse rate six-months post-P2-VP8*

Of 33 who shed, IgA serum anti-P2-VP8-P[8] seroresponse six months after the third injection was shown in 29 (87.88%), compared with 77/108 who did not shed (71.30%; p=0.054). The MH test indicated that treatment allocation was not an effect modifier in this relationship (p=0.382).

**Table** 3.5 shows the final adjusted logistic regression model, derived from backward selection, displaying the relationship between the IgA seroresponse rate to P[8] at the six-month time point and RV shedding. According to this model, IgA seroresponse rate to P[8] was not significant (p=0.069) at the 5% level. Treatment allocation was the only significant covariate (p=0.005). No confounders which modified the OR by 10% were identified. Post-estimation goodness of fit tests indicated that the model was well specified. The multivariate model indicates that the association between adjusted IgA seroresponse to P[8] six months post-P2-VP8 vaccination and RV shedding was marginally significant (AOR=2.92; 95% CI: [0.92-9.30]; p=0.069). Seroreresponse was more frequent amongst RV shedders than non-shedders.

**Table 3.6: Final logistic regression model showing the relationship between the anti-P[8] IgA seroresponse six months after receiving the third injection and RV shedding, as determined by ELISA (n=138).**

| Variables | AOR | Std. Err. | z | P>z | 95% CI |
|---|---|---|---|---|---|
| IgA to P[8] six-months post-P2-VP8 seroresponse | | | | | |
| Positive | 2.92 | 1.73 | 1.82 | 0.069 | [0.92-9.30] |
| Treatment allocation | | | | | |
| Vaccine | 0.31 | 0.13 | -2.76 | 0.006 | [0.13-0.71] |
| *Constant* | 0.26 | 0.15 | -2.34 | 0.019 | [0.08-0.80] |

Table notes: IgA to P[8] six-months post-P2-VP8 seroresponse is a categorical variable which is positive if the magnitude of the IgA antibody response to the P2-VP8 vaccine antigen measured six-months after receiving three doses of P2-VP8 vaccine or placebo is four-fold greater than the same response measured at baseline. Baseline refers to the analysis of sera conducted at baseline (enrolment); six-months post-P2-VP8 refers to the analysis of sera conducted six months after receiving three doses of parenteral P2-VP8-P[8] vaccine or placebo; Std. Err: standard error; CI: confidence interval.

#### *3.2.3.2 IgA Antibodies to Whole RV Lysate*

*a. Magnitude one-month post-P2-VP8*

The difference (1.32 titres) in the magnitude of IgA antibodies to whole RV lysate at baseline between RV shedders and non-shedders was not significant (p=0.2639). In comparison to baseline measurements, the difference (4.39 titres) was slightly larger when measured **one month** after receiving three doses of P2-VP8-P[8] vaccine or placebo. Infants who did not shed (12.36 GMT: 95% CI 9.78-15.62) had a higher IgA immune response to whole RV lysate than infants who did shed RV (7.79 GMT: 95% CI 7.35-8.24). The Wilcoxon Rank-Sum test indicates that this difference in IgA antibodies to whole RV lysate between RV shedders and non-shedders, one-month post-P2-VP8 vaccination, while small, is significant (p=0.041). The frequency of RV shedding in stools is lower in infants who have higher post-P2-VP8 IgA to whole RV lysate immune responses. The MH test confirmed that treatment allocation was not an effect modifier in this relationship (p=0.226). However, unadjusted logistic regression indicated that there was insufficient evidence to reject the null hypothesis that there was no association between one-month post-P2-VP8-P[8] vaccine IgA antibodies to whole RV lysate and RV shedding (p=0.313).

Table 3.7 shows the final adjusted logistic regression model, derived from backward selection, displaying the relationship between IgA antibody titres (**one-month** post-P2-VP8 vaccination) to whole RV lysate and RV shedding. Treatment allocation was the only significant covariate (p=0.02), however the magnitude of the IgA response to P[8] at baseline was marginally significant when conducting the likelihood ratio test (p=0.0605) to determine which covariates should be included in the final model. No other confounders which modified the OR by more than 10% were identified. The odds ratio indicates that for every one unit increase in antibody titres to whole RV lysate one-month post-P2-VP8 vaccination, there is a 0.05% decrease in the odds of being a shedder (AOR=0.95; p=0.314). However, this is not statistically significant. Therefore, despite the earlier

indications from the unadjusted analysis, an association between the magnitude of the IgA antibody response to whole RV lysate, measured post-P2-VP8 vaccination, and RV shedding in infant stools, derived from Rotarix administration, cannot be detected after adjusting for treatment arm.

**Table 3.7: Final logistic regression model showing the relationship between the magnitude of the adjusted IgA antibody response to whole RV lysate one-month post-P2-VP8 vaccination and shedding of RV in stools, as determined by ELISA (n=143).**

| Variables | AOR | Std. Err. | z | P>z | 95% CI |
|---|---|---|---|---|---|
| IgA to whole RV lysate one-month post-P2-VP8 (titres) | 0.95 | 0.07 | -0.82 | 0.410 | [0.82-1.08] |
| Treatment allocation<br>Vaccine | 0.36 | 0.16 | -2.36 | 0.018 | [0.15-0.84] |
| IgA to P[8] at baseline (titres) | 1.04 | 0.03 | 1.37 | 0.171 | [0.98-1.09] |
| *Constant* | 0.77 | 0.49 | -41 | 0.681 | [0.22-2.69] |

Table notes: IgA to whole RV lysate refers to the magnitude of the IgA antibody response to whole rotavirus lysate; IgA to P[8] refers to the magnitude of the IgA antibody response to the P2-VP8 vaccine antigen; one-month post-P2-VP8 refers to the analysis of sera conducted one month after receiving three doses of parenteral P2-VP8-P[8] vaccine or placebo; six-months post-P2-VP8 refers to the analysis of sera conducted six months after receiving three doses of parenteral P2-VP8-P[8] vaccine or placebo; baseline refers to the analysis of sera conducted at baseline (enrolment); AOR: Adjusted Odds Ratio; Std. Err: Standard Error; CI: Confidence Interval.

*b. Magnitude six-months post-P2-VP8 vaccination*

In comparison to measurements at baseline and at the one-month time point, the difference (-148 titres) in the magnitude of IgA antibodies to whole RV lysate between RV shedders and non-shedders is significant (p=0.006) and much larger when measured **six months** after receiving three doses of the P2-VP8-P[8] vaccine or placebo. Infants who did not shed (81.44 GMT: 95% CI 62.36-106.36) had a lower IgA immune response to whole RV lysate than infants who shed RV (229.82 GMT: 95% CI 142.15-371.55). The MH test confirmed that treatment allocation was not an effect modifier in this relationship (p=0.8268).

Unadjusted logistic regression indicated that for every 2.72[3]-fold increase in log IgA titres to whole RV lysate, there is a 1.71-fold (95% CI 1.25-2.33) increase in the odds of having shed RV in stools post challenge (p=0.001).

Table 3.8 shows the final adjusted logistic regression model, derived from backward selection, displaying the relationship between IgA antibody titres (six months post-P2-VP8 vaccination) to whole RV lysate and RV shedding. Treatment allocation was the only significant covariate (p=0.028), however, the likelihood ratio test indicated that model fit was improved through the inclusion of IgA antibodies to P[8] and whole RV lysate at baseline (P-values for the likelihood ratio tests were p=0.0548, and p<0.001, respectively) in comparison to more parsimonious models.

[3] Natural log was used in transformation which means base is equal to *e (2.72)*.

IgA antibodies to P[8] modified the OR for IgA to whole RV lysate at the six-month time point by 32%, but no other confounders which modified the OR by more than 10% were identified. The odds ratio indicates that for every 2.72[4]-fold increase in antibody titres to whole RV lysate six-months post-P2-VP8 vaccination, holding all other variables constant, there was a 1.96-fold increase in the odds of having shed vaccine virus post-challenge (AOR=1.96; 95% CI: [1.37-2.82]; p<0.001). Those who had high IgA titres to whole RV lysate six months after receiving three doses of P2-VP8-P[8] vaccine or placebo, were more likely to have shed RV in stools following Rotarix administration, after adjusting for treatment allocation.

**Table 3.8: Final logistic regression model showing the relationship between the magnitude of the adjusted IgA antibody response to whole RV lysate six-months post-P2-VP8 vaccination and shedding of RV in stools, as determined by ELISA (n=138).**

| Variables | AOR | Std. Err. | Z | P>z | 95% CI |
|---|---|---|---|---|---|
| IgA to whole RV lysate six-months post-P2-VP8 (log titres) | 1.96 | 0.36 | 3.67 | <0.001 | [1.37-2.82] |
| Treatment allocation | | | | | |
| Vaccine | 0.34 | 0.17 | -2.20 | 0.028 | [0.13-0.89] |
| IgA to P[8] at baseline (titres) | 1.04 | 0.03 | 1.41 | 0.159 | [0.98-1.10] |
| IgA to whole RV lysate at baseline (titres) | 0.94 | 0.06 | -0.87 | 0.385 | [0.83-1.07] |
| *Constant* | 0.03 | 0.03 | -3.12 | 0.002 | [0.00-0.27] |

Table notes: IgA to whole RV lysate refers to the magnitude of the IgA antibody response to whole rotavirus lysate; IgA to P[8] refers to the magnitude of the IgA antibody response to the P2-VP8 vaccine antigen; one-month post-P2-VP8 refers to the analysis of sera conducted one month after receiving three doses of parenteral P2-VP8-P[8] vaccine or placebo; six-months post-P2-VP8 refers to the analysis of sera conducted six months after receiving three doses of parenteral P2-VP8-P[8] vaccine or placebo; baseline refers to the analysis of sera conducted at baseline (enrolment); AOR: Adjusted Odds Ratio; Std. Err: Standard Error; CI: Confidence Interval.

### *c. Seroresponse one-month post-P2-VP8 vaccination*

Anti-whole RV lysate IgA seroresponse, one month after receiving the third injection, was low overall (11/147; 7.48%). None of the infants who shed RV following Rotarix administration showed IgA seroresponse to the whole RV lysate, compared to 11 (9.91%) of infants who did not shed (p=0.064). There were too few observations in the sub-categories for this analysis to be appropriately powered (N=11) or to run a multivariate analysis.

### *d. Seroresponse six-months post-P2-VP8 vaccination*

Of 31 infants who shed RV post-challenge, IgA seroresponse to whole RV lysate six months after receiving the third P2-VP8 injection/placebo was shown in 28 (90.32%), compared to 73/107 (68.22%) who did not shed (p=0.014). IgA anti-whole RV lysate seroresponse was significantly more frequent in infants who shed RV compared to infants who did not shed post-challenge. The MH test indicated that treatment allocation was not an effect modifier in this relationship (p=0.737).

[4] Interpretation of a log transformed predictor variable so that the odds ratio is associated with a $b$-fold increase in the predictor where $b$ is equal to the base of the logarithm used ($e = 2.72$).

Unadjusted logistic regression indicated that infants who showed a positive seroresponse to IgA whole RV lysate, six months after receiving three doses of P2-VP8-P[8] vaccine or placebo, were 4.35 times more likely to have shed RV after Rotarix administration (OR=4.35; 95% CI: [1.24-15.30]; p=0.022) compared to those who showed a negative seroresponse.

Table 3.9 shows the final adjusted logistic regression model, derived from backward selection, displaying the relationship between IgA antibody seroresponse rate (six months post-P2-VP8 vaccination) to whole RV lysate and RV shedding post Rotarix administration. Treatment allocation was the only significant covariate (p=0.004), however, the likelihood ratio test indicated that model fit was improved through the inclusion of IgA antibodies to P[8] at baseline and whole RV lysate one month post-P2-VP8 (p-values for the likelihood ratio tests were p=0.025, and p<0.001, respectively) in comparison to more parsimonious models. Gender was identified as a confounder which modified the OR for IgA to whole RV lysate at the six-month time point by 12.21%, but no other confounders which modified the OR by more than 10% were identified. The odds ratio indicates that infants who seroconverted on the basis of IgA antibodies to whole RV lysate at the six-month time point were 10.79 (95% CI [2.25-51.85]) times more likely to have shed RV in stools following Rotarix administration, holding all other relevant variables constant (p=0.003).

**Table 3.9: Final logistic regression model showing the relationship between the IgA antibody seroresponse to whole RV lysate six-months post-P2-VP8 and shedding of RV in stools, as determined by ELISA (n=138).**

| Variables | AOR | Std. Err. | z | P>z | 95% CI |
|---|---|---|---|---|---|
| IgA to whole RV lysate six-months post-P2-VP8 seroresponse rate | | | | | |
| Positive | 10.79 | 8.64 | 2.97 | 0.003 | [2.25-51.85] |
| Treatment allocation | | | | | |
| Vaccine | 0.24 | 0.12 | -2.85 | 0.004 | [0.09-0.64] |
| Gender | | | | | |
| Female | 0.57 | 0.27 | -1.18 | 0.238 | [0.22-1.45] |
| IgA to P[8] at baseline (titres) | 1.05 | 0.03 | 1.56 | 0.118 | [0.99-1.12] |
| IgA to whole RV lysate one-month post-P2-VP8 (titres) | 0.95 | 0.06 | -0.80 | 0.424 | [0.83-1.08] |
| *Constant* | 0.17 | 0.16 | -1.83 | 0.067 | [0.02-1.13] |

Table notes: IgA to whole RV lysate six-months post-P2-VP8 seroresponse is a categorical variable which is positive if the magnitude of the IgA antibody response to whole RV lysate measured six-months after receiving three doses of P2-VP8 vaccine or placebo is four-fold greater than the same response measured at baseline; IgA to P[8] refers to the magnitude of the IgA antibody response to the P2-VP8 vaccine antigen; baseline refers to the analysis of sera conducted at baseline (enrolment); one-month post-P2-VP8 refers to the analysis of sera conducted one month after receiving three doses of parenteral P2-VP8-P[8] vaccine or placebo; six-months post-P2-VP8 refers to the analysis of sera conducted six months after receiving three doses of parenteral P2-VP8-P[8] vaccine or placebo; AOR: adjusted odds ratio; Std. Err: standard error; CI: confidence interval.

### 3.2.4 IgG serum immune responses to P[8] vaccine antigen

The bivariate analysis, which tested for an association between RV shedding and IgG antibodies to the P2-VP8-P[8] sub-unit vaccine antigen is shown in Table 3.10.

**Table 3.10: Differences in magnitude and seroresponse rate of IgG antibody responses to NRRV P2-VP8 sub-unit vaccine antigen, measured at baseline, one and six months after receiving three doses of NRRV P2-VP8 sub-unit vaccine or placebo (post-P2-VP8), between negative and positive RV shedders, as determined by ELISA (N=147).**

| IgG to P[8] antibody response | Shedder (ELISA Positive) | Non-shedder (ELISA Negative) | P-value |
|---|---|---|---|
| **Baseline** | | | |
| Magnitude, GMT [95% CI] | 84.34 [53.55-132.83] | 122.93 [93.79-161.11] | 0.110 |
| **One-month post-P2-VP8** | | | |
| Magnitude, GMT [95% CI]† | 496.71 [158.27-1558.88] | 2423.51 [1521.16-3861.14] | 0.181 |
| **Seroresponse, n (col%)** | | | **0.005** |
| Negative | 17 (50.00) | 28 (24.78) | |
| Positive | 17 (50.00) | 85 (75.22) | |
| mAb adjusted magnitude, GMT [95% CI]† | 2098.36 [671.25-6559.57] | 10302.38 [6445.75-16466.51] | 0.140 |
| **mAb adjusted seroresponse, n (col%)** | | | **0.004** |
| Negative | 16 (47.06) | 25 (22.12) | |
| Positive | 18 (52.94) | 88 (77.88) | |
| **Six-months post-P2-VP8** | | | |
| Magnitude, GMT [95% CI]† | 1122.72 [655.24-1923.71] | 825.68 [605.58-1125.77] | 0.530 |
| Seroresponse, n (col%) | | | 0.489 |
| Negative | 9 (27.27) | 37 (33.94) | |
| Positive | 24 (72.73) | 72 (66.06) | |
| mAb adjusted magnitude, GMT [95% CI]† | 9067.75 [5295.32-15527.68] | 6687.45 [4902.83-9121.65] | 0.532 |
| mAb adjusted seroresponse, n (col%) | | | 0.561 |
| Negative | 3 (9.09) | 14 (12.84) | |
| Positive | 30 (90.91) | 95 (87.16) | |

Table notes: Wilcoxon Rank Sum was conducted for continuous variables unless variance was equal and a t-test was performed (indicated by a dagger†). Pearson's chi-squared or fisher's exact (indicated by an asterisk*) were conducted for categorical variables. IgG to P[8] refers to the IgG antibody response to the P2-VP8 vaccine antigen; one-month post-P2-VP8 refers to the analysis of sera conducted one-month after receiving three doses of parenteral P2-VP8-P[8] vaccine or placebo; six-months post-P2-VP8 refers to the analysis of sera conducted six months after receiving three doses of parenteral P2-VP8-P[8] vaccine or placebo; mAb adjusted refers to titres which have been adjusted for the decay of maternal IgG antibodies in infants as they get older; seroresponse is a categorical variable which is positive if the magnitude of the IgG antibody response to P2-VP8 vaccine antigen measured either one- or six-months after receiving three doses of P2-VP8 vaccine or placebo is four-fold greater than the same response measured at baseline. Abbreviations: RV: rotavirus; IgA: immunoglobulin A; GMT: geometric mean titres; n: number of observations; col%: column percentages; RV: rotavirus; CI: confidence interval; ELISA: enzyme-linked immunosorbent assay.

The differences in the magnitude of the IgG immune response between RV shedders and non-shedders is depicted graphically in Figure 3.3.

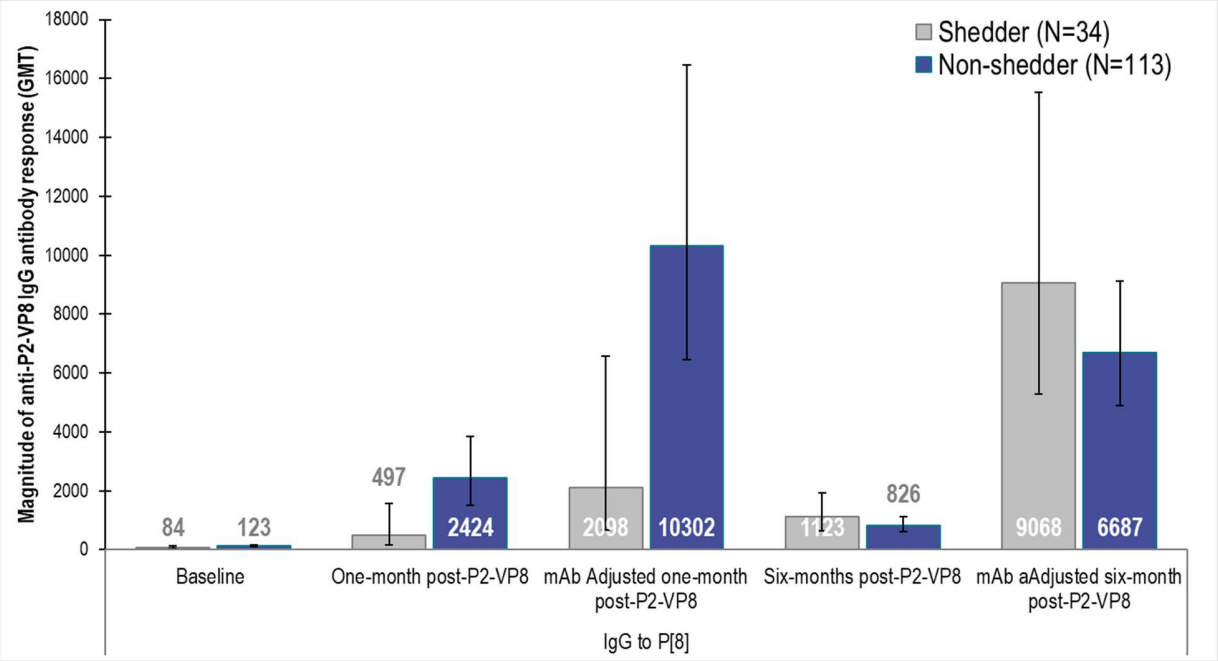



**Figure 3.3: Difference in magnitude of IgG antibody response (GMT ± 95% CI) to P2-VP8-P[8] sub-unit vaccine antigen between infants who shed RV (shedder) and infants who do not shed RV (non-shedder) after administration of Rotarix, as determined by ELISA (N=147).** Post-P2-VP8 refers to the analysis of sera conducted one or six months after receiving three doses of parenteral P2-VP8-P[8] vaccine or placebo; mAb adjusted refers to titres which have been adjusted for the decay of maternal IgG antibodies in infants as they get older; IgG antibody response was measured at baseline and one and six months after receiving three doses of P2-VP8 sub-unit vaccine (post-P2-VP8). Abbreviations: IgG to P[8]: antibody response to P2-VP8 sub-unit vaccine antigen; GMT: geometric mean titres. Asterisk indicates significance at the 5% level* or at the 1% level**.

*a. Magnitude at baseline*

The magnitude of the IgG titres to P[8] at baseline was smaller in infants who shed RV (84.34 GMT; 95% CI 53.55-132.83) compared to infants who did not shed RV (122.93 GMT; 95% CI 93.79-161.11), but the difference (38.49 titres) was not significant (p=0.110). While insignificant, the direction of this difference indicates that infants who have higher IgG P[8] titres at baseline may be less likely to shed RV in stools. The MH test was not able to detect effect modification by treatment allocation on the relationship between RV shedding and IgG antibody titres to P[8] at baseline (p=0.304).

*b. Magnitude one-month post-P2-VP8*

The difference (1872.10 titres) in the magnitude of the IgG antibody titres to P[8], without adjustment for maternal antibodies, between shedders (496.71 GMT; 95% CI 158.27-1558.8) and (2423.51 GMT; 95% CI 1521.16-3861.14) non-shedders of RV in stools was much larger when measured one month after receiving three doses of P2-VP8-P[8] vaccine or placebo. Despite this difference, the T-test indicated that the magnitude of IgG antibodies to P[8] post-vaccination did

not differ significantly between shedders and non-shedders (p= 0.1806). The MH test confirmed that treatment allocation was not an effect modifier in this relationship (p=0.2918).

A similar trend was observed for IgG antibody titres to P[8] adjusted for decay of maternal antibodies over time. The difference in the magnitude of response between shedders and non-shedders of RV is much larger (8204.02 GMT) and the magnitude of the antibody response is much higher when compared to baseline or unadjusted post-P2-VP8 vaccination titres. However, the difference is not significant (p=0.1399). The MH test confirmed that treatment allocation was not an effect modifier in this relationship (p=0.3088).

*c. Magnitude six-months post-P2-VP8*

The difference (-297.04 titre) in the magnitude of the IgG antibody response to P[8], without adjustment for maternal antibodies, between shedders (1122.72 GMT; 95% CI 655.24-1923.71) and non-shedders (825.68 GMT; 95% CI 605.58-1125.77) was not much larger when measured six month after receiving three doses of P2-VP8-P[8] vaccine or placebo. The T-test indicated that the magnitude of IgG antibodies to P[8] six months post-P2-VP8 did not differ significantly between shedders and non-shedders (p= 0.530). The MH test confirmed that treatment allocation was not an effect modifier in this relationship (p=0.589).

Similarly, when considering the magnitude of adjusted IgG antibody titres to P[8], the difference in GMT (-2380.30) between shedders (9067.75 GMT; 95% CI 5295.32-15527.68) and non-shedders (6687.45 GMT; 95% CI 4902.83-9121.65) of RV was larger than the unadjusted response but the difference was not significant (p=0.532). The MH test confirmed that treatment allocation was not an effect modifier in this relationship (p=0.654).

*d. Seroresponse rate one-month post-P2-VP8*

Of 34 infants who shed, unadjusted IgG serum anti-P2-VP8-P[8] seroresponse one month after the third injection was shown in 17 (50.00%), compared with 85/113 (75.22%) who did not shed. Unadjusted IgG serum anti-P2-VP8-P[8] seroresponse was significantly higher in those who did not shed (p=0.005). Infants who have a positive IgG seroresponse to P[8] were 67% less likely to shed RV in stools than infants who had a negative IgG seroresponse to P[8] (OR=0.33; p=0.006). The MH test confirmed that treatment allocation was not an effect modifier in this relationship (p=0.3160).

Similarly, when adjusting for the decay of maternal IgG antibodies, there was an association between the seroresponse rate of IgG antibodies to P[8] and RV shedding. Of 34 infants who shed, adjusted IgG serum anti-P2-VP8-P[8] seroresponse one month after the third injection was shown in 18 (52.94%), compared with 88/113 (77.88%) who did not shed. Adjusted IgG serum anti-P2-VP8-P[8] seroresponse was significantly higher in those who did not shed (p=0.004). Infants who seroconverted were 68% less likely to shed RV than infants who did not seroconvert (OR=0.32; p=0.006). The MH test confirmed that treatment allocation was no an effect modifier in this relationship (p=0.554). Despite the indication of a significant relationship in the bivariate tests, a multivariate analysis could not be conducted due to the very low number of observations present in the sub-categories.

*e. Seroresponse rate six months post-P2-VP8*

Of 33 infants who shed, unadjusted IgG serum anti-P2-VP8-P[8] seroresponse six months after the third injection was shown in 24 (72.73%), compared with 72/109 (66.06%) who did not shed but this difference was not statistically significant(p=0.489). The MH test confirmed that treatment allocation was not an effect modifier in this relationship (p=0.668).

Of 33 infants who shed, adjusted IgG serum anti-P2-VP8-P[8] seroresponse six months after the third injection was shown in 30 (90.91%), compared with 95/109 (87.61%) who did not shed. Adjusted IgG serum anti-P2-VP8-P[8] seroresponse was not significantly higher in those who shed (p=0.561).The MH test confirmed that treatment allocation was not an effect modifier in this relationship (p=0.738).

**Table 3.11: Final logistic regression model showing the relationship between IgG to p[8] seroresponse rate six-months post-P2-VP8 and RV shedding, as determined by ELISA (n=138).**

| Variables | AOR | Std. Err | z | P>\|z\| | 95% CI |
|---|---|---|---|---|---|
| mAb adjusted IgG to P[8] six-months post P2-VP8 seroresponse | | | | | |
| Positive | 3.53 | 2.83 | 1.57 | 0.116 | [0.73-17.02] |
| Treatment allocation | | | | | |
| Vaccine | 0.19 | 0.10 | -3.32 | 0.001 | [0.07-0.51] |
| IgG to P[8] at baseline (titres) | 1.00 | 0.00 | -0.55 | 0.582 | [0.99-1.00] |
| IgA to whole RV lysate at baseline (titres) | 0.99 | 0.01 | -0.91 | 0.364 | [0.97-1.01] |
| Platelets (count) at baseline | 1.00 | 0.00 | 1.06 | 0.290 | [0.99-1.00] |
| *Constant* | *0.14* | *0.16* | *-1.78* | 0.075 | [0.02-1.22] |

Table notes: IgG to to P[8] six-months post-P2-VP8 seroresponse is a categorical variable which is positive if the magnitude of the IgG antibody response to P[8] measured six-months after receiving three doses of P2-VP8 vaccine or placebo is four-fold greater than the same response measured at baseline; mAb adjusted refers to titres which have been adjusted for the decay of maternal IgG antibodies in infants as they get older; IgG to P[8] refers to the magnitude of the IgG antibody response to the P2-VP8 vaccine antigen; IgA to whole RV lysate refers to the magnitude of the IgA antibody response to the whole rotavirus lysate; baseline refers to the analysis of sera conducted at baseline (enrolment); one-month post-P2-VP8 refers to the analysis of sera conducted one month after receiving three doses of parenteral P2-VP8-P[8] vaccine or placebo; six-months post-P2-VP8 refers to the analysis of sera conducted six months after receiving three doses of parenteral P2-VP8-P[8] vaccine or placebo; AOR: adjusted odds ratio; Std. Err: standard error; CI: confidence interval.

Table 3.11 shows the final adjusted logistic regression model, derived from backward selection, displaying the relationship between the adjusted IgG seroresponse rate to P[8] six months post-P2-VP8 and shedding. Treatment allocation, clinical haematology results (white blood cells, platelets, eosinophils, neutrophils, basophils, monocytes, lymphocytes), baseline IgG and IgA serum responses to P4, P6, and P[8], baseline IgA serum responses to whole RV lysate, and baseline NAb responses to RV strain Wa were all considered as potential covariates. Factors which modified the OR by more than 10% were identified and included in the final model; this included the magnitude of the IgG response to P[8] at baseline, the IgA response to whole RV lysate at baseline, and platelet count at baseline. The likelihood ratio test for a model which included an interaction term between the IgG seroresponse rate to P[8] and treatment allocation could not be conducted due to insufficient observations. The adjusted IgG seroresponse rate to P[8] was not significantly associated with RV shedding (AOR=3.53; 95% CI: [0.73-17.02]; p=0.116). The postestimation goodness of fit command (p=0.4184) indicates that there is insufficient evidence to reject the null hypothesis that the model is well-fitted.

### 3.2.5 Neutralising antibody responses

The bivariate analysis, which tested for an association between RV shedding and NAb immune responses to P[8], P[6], and P[4] RV strains (Wa (G1P[8]), 89-12 (G1P[8]), DS-1 (G2P[4]), and 1076 (G2P[6]), respectively) is shown in Table 3.12.

**Table 3.12: Differences in magnitude and seroresponse rate of NAb responses to different strains of RV measured at baseline and one month and six months after receiving three doses of P2-VP8 sub-unit vaccine or placebo (post-P2-VP8) between negative and positive RV shedders, as determined by ELISA.**

| Neutralising antibody response | Shedder (ELISA Positive) N=34 | Non-shedder (ELISA Negative) N=113 | P-value |
|---|---|---|---|
| **Neutralising antibody response to RV strain Wa** | | | |
| ***Baseline*** | | | |
| Magnitude, GMT [95% CI] | 66.79 [46.42-96.09] | 97.43 [78.78-120.49] | 0.046 |
| ***One-month post-P2-VP8*** | | | |
| Magnitude, GMT [95% CI] | 45.67 [24.68-84.51] | 110.41 [84.38-144.47] | 0.024 |
| Seroresponse, n (col%) | | | 0.649 |
| Negative | 28 (82.35) | 89 (78.76) | |
| Positive | 6 (17.65) | 24 (21.24) | |
| mAb adjusted magnitude, GMT [95% CI] | 330.35 [179.4-608.32] | 795.61 [601.9-1051.67] | 0.023 |
| mAb adjusted seroresponse, n (col%) | | | 0.067 |
| Negative | 17 (50.00) | 37 (32.74) | |
| Positive | 17 (50.00) | 76 (67.26) | |
| ***Six-months post-P2-VP8*** | | | |
| Magnitude, GMT [95% CI]† | 205.40 [133.02-317.16] | 82.07 [64.11-105.06] | <0.001 |
| Seroresponse, n (col%) | | | 0.003 |
| Negative | 20 (60.61) | 92 (84.40) | |
| Positive | 13 (39.39) | 17 (15.60) | |
| mAb adjusted magnitude, GMT [95% CI] | 1377.22 [893.15-2123.66] | 551.45 [430.67-706.10] | <0.001 |
| mAb adjusted seroresponse, n (col%) | | | 0.008 |
| Negative | 5 (15.15) | 44 (40.37) | |
| Positive | 28 (84.85) | 65 (59.63) | |
| **Neutralising antibody response to RV strain 89-12** | | | |
| ***Baseline*** | 83.51 [54.37-128.28] | 127.27 [101.71-159.23] | 0.452 |
| Magnitude, GMT [95% CI] | 83.51 [54.37-128.28] | 127.27 [101.71-159.23] | 0.452 |
| ***One-month post-P2-VP8*** | | | |
| Magnitude, GMT [95% CI] | 73.81 [37.7-144.47] | 183.99 [138.9-243.73] | 0.030 |
| Seroresponse, n (col%) | | | 0.403 |
| Negative | 26 (76.47) | 78 (69.03) | |
| Positive | 8 (23.53) | 35 (30.97) | |
| mAb adjusted magnitude, GMT [95% CI] | 468.47 [240.93-910.9] | 1162.58 [871.57-1550.76] | 0.027 |
| mAb adjusted seroresponse, n (col%) | | | 0.084 |
| Negative | 17 (50.00) | 38 (33.63) | |
| Positive | 17 (50.00) | 75 (66.37) | |
| ***Six-months post-P2-VP8*** | | | |
| Magnitude, GMT [95% CI]† | 323.51 [208.23-502.61] | 123.39 [97.37-156.35] | <0.001 |
| Seroresponse, n (col%) | | | 0.012 |
| Negative | 20 (60.61) | 89 (81.65) | |
| Positive | 13 (39.39) | 20 (18.35) | |
| mAb adjusted magnitude, GMT [95% CI] | 2217.88 [1430.50-3438.67] | 847.71 [668.80-1074.48] | <0.001 |
| mAb adjusted seroresponse, n (col%) | | | 0.020 |
| Negative | 5 (15.15) | 40 (36.70) | |
| Positive | 28 (84.85) | 69 (63.30) | |

| Neutralising antibody response | Shedder (ELISA Positive) N=34 | Non-shedder (ELISA Negative) N=113 | P-value |
|---|---|---|---|
| **Neutralising antibody response to RV strain 1076[5]** | | | |
| ***Baseline*** | | | |
| Magnitude, GMT [95% CI] | 42.01 [29-60.87] | 51 [41.08-63.31] | 0.322 |
| ***One-month post-P2-VP8*** | | | |
| Magnitude, GMT [95% CI] | 15.11 [10.1-22.6] | 19 [15.42-23.41] | 0.306 |
| Seroresponse, n (col%) | | | 0.337 |
| Negative | 34 (100.0) | 110 (97.3) | |
| Positive | 0 (0.0) | 3 (2.7) | |
| mAb adjusted magnitude, GMT [95% CI] | 71.43 [47.93-106.44] | 89.41 [72.38-110.45] | 0.382 |
| mAb adjusted seroresponse, n (col%) | | | 0.812 |
| Negative | 28 (82.3) | 91 (80.5) | |
| Positive | 6 (17.7) | 22 (19.5) | |
| **Neutralising antibody response to RV strain ds1** | | | |
| ***Baseline*** | | | |
| Magnitude, GMT [95% CI] | 66.72 [44.57-99.87] | 69.5 [55.89-86.43] | 0.985 |
| ***One-month post-P2-VP8*** | | | |
| Magnitude, GMT [95% CI] | 15.78 [10.71-23.26] | 23.38 [18.74-29.16] | 0.159 |
| Seroresponse, n (col%) | | | 0.545 |
| Negative | 32 (94.1) | 109 (96.5) | |
| Positive | 2 (5.9) | 4 (3.5) | |
| mAb adjusted magnitude, GMT [95% CI] | 129.27 [88.28-189.28] | 191.1 [152.42-239.6] | 0.203 |
| mAb adjusted seroresponse, n (col%) | | | 0.153 |
| Negative | 28 (82.3) | 79 (69.9) | |
| Positive | 6 (17.7) | 34 (30.1) | |

Table notes: Seroresponse is a categorical variable which is positive if the magnitude of the neutralising antibody response to one of the RV strains, measured six-months after receiving three doses of P2-VP8 vaccine or placebo, is four-fold greater than the same response measured at baseline; mAb adjusted refers to titres which have been adjusted for the decay of maternal antibodies in infants as they get older; Magnitude refers to the magnitude of the neutralising antibody response to the RV strain; baseline refers to the analysis of sera conducted at baseline (enrolment); one-month post-P2-VP8 refers to the analysis of sera conducted one month after receiving three doses of parenteral P2-VP8-P[8] vaccine or placebo; six-months post-P2-VP8 refers to the analysis of sera conducted six months after receiving three doses of parenteral P2-VP8-P[8] vaccine or placebo; GMT: geometric mean titres; RV: rotavirus; n: number of observations; col%: column percentage; mAb: maternal antibody; CI: confidence interval; ELISA: enzyme-linked immunosorbent assay.

The differences in the magnitude of the NAb response to RV strains Wa, DS-1, 1076 and 8912 between RV shedders and non-shedders are depicted graphically in Figure 3.4.

[5] NAb to RV strain 1076 and Ds1 were not measured six months post-P2-VP8.

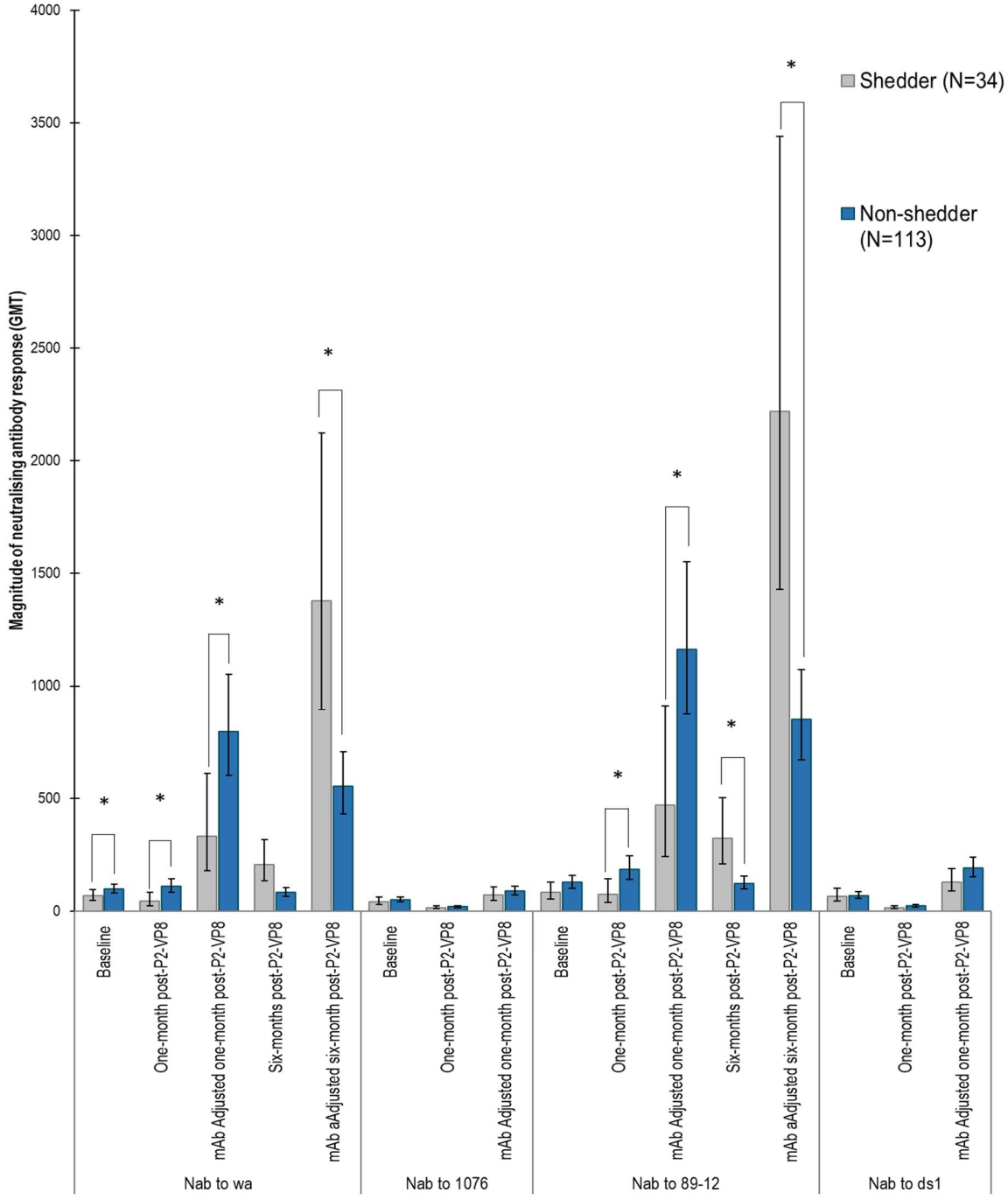



**Figure 3.4: Difference in magnitude of NAb responses (GMT ± 95% CI) to rotavirus strains Wa, 8912, 1076 and DS-1 between infants who shed RV (shedder) and infants who do not shed RV (non-shedder), as determined by ELISA (N=147). *Indicates that difference was significant at the 5% level as determined by Wilcoxon Rank Sum or T-test. Post-P2-VP8 refers to the analysis of sera conducted one month or six months after receiving three doses of parenteral P2-VP8-P[8] vaccine or placebo.** Post-P2-VP8 refers to the analysis of sera conducted one or six months after receiving three doses of parenteral P2-VP8-P[8] vaccine or placebo; mAb adjusted refers to titres which have been adjusted for the decay of maternal antibodies in infants as they get older; Nab refers to neutralising antibody response to RV strains; antibody response was measured at baseline and one and six months after receiving three doses of P2-VP8 sub-unit vaccine. Abbreviations: Nab: neutralising antibody response; GMT: geometric mean titres. Asterisk indicates significance at the 5% level* or at the 1% level**.

#### *3.2.5.1 Neutralising antibody to RV strain Wa*

*a. Magnitude one-month post-P2-VP8*

There was a significant difference (30.64) in the GMTs of NAbs to RV strain Wa at baseline between shedders and non-shedders (p=0.046). Infants who shed RV (66.79 GMT; 95% CI 46.42-96.09) had significantly lower NAb responses to RV strain Wa compared to infants who did not shed RV (97.43 GMT; 95% CI 78.78-120.49).

The difference (64.71) in GMTS was slightly larger when measured one month after receiving three doses of P2-VP8-P[8] vaccine or placebo. Infants who shed RV in stools had a lower NAb titre (45.67 GMT; 95% CI 24.68-84.51) than infants who did not shed RV (110.41 GMT; 95% CI 84.38-144.47; p=0.024). The MH test confirmed that treatment allocation was not an effect modifier in this relationship (p=0.4068). Unadjusted logistic regression indicated that for a 100-fold increase in the NAb titre to RV strain Wa, there would be a 23.94% decrease in the odds of shedding RV in stools (OR=0.76, 95% CI: [0.58-0,99]; p=0.046).

When considering the NAb titres, previously adjusted for the decay of maternal antibodies, the difference in GMT (465.26) is much larger than the unadjusted difference measured one month after receiving three doses of P2-VP8-P[8] vaccine or placebo. Infants who shed RV had a significantly (p=0.023) lower NAb GMTs (330.35; 95% CI [179.4-608.32]) than infants who did not shed RV (795.61; 95% CI [601.9-1051.67]). The multivariate analysis indicated that were no other significant covariates and no other confounders which modified the OR by more than 10% were identified. Results indicated that for every 100-fold increase in mAb adjusted NAb titres to RV strain Wa there was a 4% decrease in the odds of shedding RV (OR=0.96, 95% CI 0.93-0.99; p=0.034).

*b. Magnitude six-months post-P2-VP8*

The difference in GMT (-123.33) was larger when measured six months after receiving three doses of P2-VP8-P[8] vaccine or placebo compared to baseline and one-month timepoints. Infants who shed RV in stools had significantly (p<0.001) **higher** NAb GMT (205.40; 95% CI 133.02-317.16) than infants who did not shed RV (82.07; 95% CI 64.11-105.06). The MH test confirmed that treatment allocation was not an effect modifier in this relationship (p=0.170).

Adjusting for decay of maternal antibodies, the difference in GMT (-1370.71) was much larger than the unadjusted difference measured one and six months after receiving the third injection.

Infants who shed RV had a significantly (p<0.001) **higher** NAb GMT (1377.22; 95% CI 893.15-2123.66) after receiving the third injection than infants who did not shed RV (551.45; 95% CI 430.67-706.10).

Multivariate backward selection process was used to investigate an adjusted logistic regression model with log-transformed NAb titres to RV strain Wa. The model shown in **Table 3.13**, below, indicates that for every 2.72-fold increase in NAb titres to RV strain Wa six months after receiving the third injection, there is a 1.93-fold increase in the odds of shedding RV post-Rotarix administration (OR=1.93, 95% CI: [1.34-2.78]; p<0.001).

**Table 3.13: Final logistic regression model showing the relationship between the magnitude of the NAb response to RV strain Wa and RV shedding, as determined by ELISA (n=138).**

| Variables | AOR | z | P>\|z\| | 95% CI |
|---|---|---|---|---|
| mAb adjusted NAb to Wa six-months post-P2-VP8 (titres) | 1.93 | 3.53 | <0.001 | [1.34-2.78] |
| Treatment allocation | | | | |
| Vaccine | 0.18 | -3.51 | <0.001 | [0.07-0.47] |
| IgA to whole RV lysate at baseline (titres) | 0.99 | -0.84 | 0.401 | [0.95-1.02] |
| Basophils at baseline (count) | 1085.35 | 1.45 | 0.147 | [0.09-1.37e+07] |
| *Constant* | *0.01* | *-3.62* | *<0.001* | *[0.00-0.13]* |

Table notes: Six-months post-P2-VP8 refers to the analysis of sera conducted six month after receiving three doses of parenteral P2-VP8-P[8] vaccine or placebo; IgA to whole RV lysate refers to the magnitude of the IgA antibody response to the whole RV lysate measured at baseline. NAb to Wa refers to the neutralising antibody response to rotavirus strain Wa; mAb adjusted refers to titres which have been adjusted for the decay of maternal antibodies in infants as they get older; AOR: adjusted odds ratio; CI: confidence interval. In the multivariate stepwise regression analysis, treatment allocation, clinical haematology results (white blood cells, platelets, eosinophils, neutrophils, basophils, monocytes, lymphocytes), baseline IgG and IgA serum responses to P[4], P[6], and P[8], baseline IgA serum responses to whole RV lysate, and baseline NAb responses to RV strain Wa were all considered as potential covariates

### *c. Seroresponse rate one-month post-P2-VP8*

Of 34 who shed, unadjusted NAb seroresponse to RV strain Wa, one month after the third injection, was shown in 6 (17.65%), compared with 24/113 (21.24%) who did not shed. This was not significant (p=0.649). Of 34 who shed, adjusted NAb seroresponse to RV strain Wa, one month after the third injection, was shown in 17 (50.00%), compared with 76/113 (67.26%) who did not shed. Adjusted NAb seroresponse to RV strain Wa was higher in those who did not shed but this was not significant (p=0.067).

Unadjusted logistic regression indicated that shedding was lower in infants who had a positive seroresponse to RV strain Wa than those who had a negative seroresponse (OR = 0.49; 95% CI [0.22-1.06]; p=0.070) but this was not significant. The MH test to determine if treatment allocation was an effect modifier in this relationship indicated there was a significant difference between the odds ratios stratified by treatment allocation (p=0.022), however there were too few observations

in the stratified categories for the separate odds ratios to be determined or for a multivariate analysis to be conducted.

*d. Seroresponse rate six-months post-P2-VP8*

The association between the seroresponse rate of NAbs to RV strain Wa six months after receiving the third injection, and RV shedding was highly significant before adjusting for covariates. Of the 33 individuals who shed RV in stools, 13 (39.39%) had a positive seroresponse to RV strain Wa six months after receiving the third injection, compared to 17 (15.60%) of 109 who did not shed RV. The difference in seroresponse rate of NAbs to RV strain Wa between shedders and non-shedders was significant (p=0.003). When considering NAb response adjusted for the decay of maternal antibodies, a similar relationship was observed. Infants who shed RV in stools had a higher seroresponse to RV strain Wa six months after receiving the third injection (28/33; 84.85%), compared to those who did not shed RV (65/109, 59.63%; p=0.008).

Unadjusted logistic regression indicated that individuals who had a positive NAb seroresponse to RV strain Wa were 3.49 times more likely to shed RV than individuals who did not (OR=3.49; p=0.018). The MH test to determine if treatment allocation was an effect modifier in this relationship indicated there was no significant difference between the odds ratios stratified by treatment allocation (p=0.705).

**Table 3.14: Final logistic regression model showing the relationship between NAb to RV strain Wa seroresponse rate six months after receiving three doses of P2-VP8-P[8] vaccine or placebo and RV shedding, as determined by ELISA (n=138).**

| Variables | AOR | Std. Err | z | P>\|z\| | 95% CI |
|---|---|---|---|---|---|
| mAb adjusted NAb to RV strain Wa six-months post-P2-VP8 seroresponse | | | | | |
| Positive | 4.14 | 2.92 | 2.88 | 0.004 | [1.69-15.65] |
| Treatment allocation | | | | | |
| Vaccine | 0.22 | 0.10 | -3.24 | 0.001 | [0.09-0.55] |
| *Constant* | *0.22* | *0.11* | *-2.96* | *0.003* | *[0.08-0.60]* |

Table notes: NAb to RV strain Wa six-months post-P2-VP8 seroresponse is a categorical variable which is positive if the magnitude of the NAb antibody response to RV strain Wa measured six-months after receiving three doses of P2-VP8 vaccine or placebo is four-fold greater than the same response measured at baseline; NAb to RV strain Wa refers to the magnitude of the NAb antibody response to RV strain Wa; mAb adjusted refers to titres which have been adjusted for the decay of maternal antibodies in infants as they get older; baseline refers to the analysis of sera conducted at baseline (enrolment); one-month post-P2-VP8 refers to the analysis of sera conducted one month after receiving three doses of parenteral P2-VP8-P[8] vaccine or placebo; six-months post-P2-VP8 refers to the analysis of sera conducted six months after receiving three doses of parenteral P2-VP8-P[8] vaccine or placebo; NAb: neutralising antibody; mAb: maternal antibody; AOR: adjusted odds ratio; Std. Err: standard error; CI: confidence interval. Treatment allocation, clinical haematology results (white blood cells, platelets, eosinophils, neutrophils, basophils, monocytes, lymphocytes), baseline IgG and IgA serum responses to P4, P6, and P[8], baseline IgA serum responses to whole RV lysate, and baseline NAb responses to RV strain Wa were all considered as covariates. There were no other significant covariates that improved the model fit or modified the odds ratio by more than 10%.

Table 3.14 shows the final adjusted logistic regression model, derived from backward selection, displaying the relationship between the NAb seroresponse to RV strain Wa six-months post-P2-VP8 vaccination and RV shedding. According to this model, the relationship was highly significant

after adjusting for treatment allocation (AOR=4.14; 95% CI: [1.69-15.65]; p=0.004). Keeping all other variables constant, infants who had a positive NAb seroresponse to RV strain Wa six-months post-P2-VP8 vaccination were 4.14 times more likely to have shed RV in stools following Rotarix administration. Post-estimation tests confirmed that the model was well-specified and there was no evidence of lack of fit.

#### *3.2.5.2 Neutralising antibody to RV strain 89-12*

*a. Magnitude one-month post-P2-VP8*

The difference (43.76) in NAb GMTs to RV strain 89-12 at baseline between shedders (83.51 GMT; 95% CI 54.37-128.28) and non-shedders (127.27 GMT; 95% CI 101.71-159.23) was not significant (p=0.452).

The difference in GMTs (110.18) was slightly larger when measured one month after receiving three doses of P2-VP8-P[8] vaccine or placebo. Infants who shed RV in stools had a significantly (p=0.030) lower NAb GMTs to RV strain 89-12 (73.81; 95% CI 37.70-144.47) than infants who did not shed RV (183.99; 95% CI 138.90-243.73). The MH test confirmed that treatment allocation was not an effect modifier in this relationship (p=0.248). Unadjusted logistic regression indicated that the relationship between log-transformed NAb response to 89-12 and RV shedding was significant (OR=0.73; 95% CI: [0.58-0.91]; p=0.006).

When adjusting for the decay of maternal antibodies, the difference in GMT (694.11) was much larger than the unadjusted difference measured one month after receiving three doses of P2-VP8-P[8] vaccine or placebo. Infants who shed RV had a lower NAb GMTs (468.47; 95% CI 240.93-910.90) than infants who did not shed RV (1162.58; 95% CI 871.57-1550.76), this difference was significant (p=0.027). Unadjusted logistic regression indicated that for every 2,72-fold increase in NAb titre to RV strain 89-12 there was a 27.12% decrease in the odds of shedding RV following Rotarix challenge (OR=0.73; 95% CI: [0.59-0.92]; p=0.007). The MH-test indicated that treatment allocation was not an effect modifier in this relationship (p=0.241).

**Table 3.15: Final logistic regression model showing the relationship between the magnitude of the adjusted NAb response to RV strain 89-12 and RV shedding one month after receiving three doses of P2-VP8-P[8] vaccine or placebo, as determined by ELISA (n=143).**

| Variables | AOR | z | P-value | 95% CI |
|---|---|---|---|---|
| mAb adjusted NAb to 89-12 one-month post-P2-VP8 (log titres) | 0.91 | -0.48 | 0.632 | [0.62 – 1.33] |
| Treatment allocation | | | | |
| Vaccine | 0.42 | -1.25 | 0.211 | [0.11-1.64] |
| *Constant* | *0.93* | *-0.08* | *0.939* | *[0.14-6.37]* |

Table notes: One-months post-P2-VP8 refers to the analysis of sera conducted one month after receiving three doses of parenteral P2-VP8-P[8] vaccine or placebo; NAb to 89-12 refers to the neutralising antibody response to rotavirus strain 89-12; mAb adjusted refers to titres which have been adjusted for the decay of maternal antibodies in infants as they get older; AOR: adjusted odds ratio; CI: confidence interval. In the multivariate stepwise regression analysis, treatment allocation, clinical haematology results (white blood cells, platelets, eosinophils, neutrophils, basophils, monocytes, lymphocytes), baseline IgG and IgA serum responses to P[4], P[6], and P[8], baseline IgA serum responses to whole RV lysate, and baseline NAb responses to RV strain Wa were all considered as potential covariates. Treatment allocation, clinical haematology results (white blood cells, platelets, eosinophils, neutrophils, basophils, monocytes, lymphocytes), baseline IgG and IgA serum responses to P4, P6, and P[8], baseline IgA serum responses to whole RV lysate, and baseline NAb responses to RV strain 89-12 were all considered as covariates. Treatment allocation was the only significant covariate and no other confounders which modified the OR by more than 10% were identified.

Table 3.15 shows the final logistic regression model, derived from backward selection, displaying the relationship between adjusted NAb titres to RV strain 89-12 and shedding. The regression model indicates that after adjusting for treatment allocation, there was no association between the one-month post-P2-VP8 adjusted NAb response to RV strain 89-12 and RV shedding in stools. Earlier indications of significance in the unadjusted analysis were due to the confounding by treatment allocation which biased the estimate away from the null hypothesis.

*a. Magnitude six-months post-P2-VP8*

The difference (-200.12 titres) when measured six months after receiving three doses of P2-VP8-P[8] vaccine or placebo was both larger than the difference at baseline and one-month post-P2-VP8, and inverted such that infants who shed RV in stools had a significantly ($p<0.001$) higher NAb response to RV strain 89-12 (323.51 GMT; 95% CI 208.23-502.61) than infants who did not shed RV (123.39 GMT; 95% CI 97.37-156.35). In contrast to the response observed one-month post-P2-VP8, where infants who have high post-P2-VP8 NAb response to RV strain 89-12 were **less likely** to have shed RV in stools (unadjusted OR), the six month post-P2-VP8 response indicated that infants who had a high NAb response to RV strain 89-12 were **more likely** to have shed RV after Rotarix challenge (OR=1.92; 95% CI: [1.33-2.77]; p=0.001). The MH test confirmed that treatment allocation was not an effect modifier in this relationship (p=0.726).

When adjusting for the decay of maternal antibodies, the difference (-1370.17 titres) is much larger than the unadjusted difference measured six months after receiving three doses of P2-VP8-P[8] vaccine or placebo. As with the unadjusted response, infants who shed RV had a significantly ($p<0.001$) higher NAb response (2217.88 GMT; 95% CI 1430.50-3438.67) than infants who did not shed RV (847.72 GMT; 95% CI 668.80-1074.48). The MH-test indicated that treatment allocation was not an effect modifier in this relationship (p=0.725). As with the unadjusted titres, for every 2.72-fold increase in NAb titres to RV strain Wa, there was a 1.92-fold increase in the odds of having shed RV following Rotarix challenge (OR=1.92; 95% CI: [1.33-2.77]; p=0.001).

Table 3.16 shows the final logistic regression model, derived from backward selection, displaying the relationship between mAb adjusted NAb titres to RV strain 89-12, six months post-P2-VP8, and RV shedding. The regression model indicates that there is a 2.09-fold increase in the odds of RV shedding for every 2.72-fold increase in mAb adjusted NAb titres to RV strain 89-12 (AOR=2.085; $p<0.001$).

**Table 3.16: Final logistic regression model showing the relationship between the magnitude of the mAb adjusted NAb response to RV strain 89-12 six months post-P2-VP8 and RV shedding, as determined by ELISA (n=138).**

| Variables | AOR | z | P-value | 95% CI |
|---|---|---|---|---|
| mAb adjusted NAb to RV strain 89-12 six-months post-P2-VP8 (log titres) | 2.09 | 3.68 | <0.001 | [1.41 – 3.09] |
| Treatment allocation | | | | |
| Vaccine | 0.22 | -3.23 | 0.001 | [0.08 - 0.55] |
| Basophils at baseline (count) | 506.04 | 1.36 | 0.173 | [0.07 – 3911677.00] |
| *Constant* | *0.00* | *-3.86* | *<0.001* | *[0.00-0.06]* |

Table notes: Six-months post-P2-VP8 refers to the analysis of sera conducted six months after receiving three doses of parenteral P2-VP8-P[8] vaccine or placebo; NAb to 89-12 refers to the neutralising antibody response to rotavirus strain 89-12; mAb adjusted refers to titres which have been adjusted for the decay of maternal antibodies in infants as they get older; AOR: adjusted odds ratio; CI: confidence interval. In the multivariate stepwise regression analysis, treatment allocation, clinical haematology results (white blood cells, platelets, eosinophils, neutrophils, basophils, monocytes, lymphocytes), baseline IgG and IgA serum responses to P4, P6, and P[8], baseline IgA serum responses to whole RV lysate, and baseline NAb responses to RV strain Wa were all considered as covariates. Treatment allocation was the only significant covariate and no other confounders which modified the OR by more than 10% were identified. Likelihood ratio tests indicated that the inclusion of basophils improved the fit of the model at the 10% level of significance compared to more parsimonious models where basophils were excluded (p=0.091).

*b. Seroresponse rate one-month post-P2-VP8*

Of 34 who shed RV, NAb response to RV strain 89-12 one month after the third injection was shown in 8 (23.53%), compared with 35 (30.97%) of 113 who did not shed. However, this was not significant (p=0.403). Similarly, when considering NAb response adjusted for the decay of maternal antibodies, RV shedding was higher in those who did not shed (75/113; 66.37%) in comparison to those who did shed (17/34; 50%) but not statistically significant (p=0.084). The unadjusted logistic regression indicated that there was some evidence that individuals who had a positive adjusted NAb response to RV strain 89-12 were 51.32% less likely to shed RV than individuals who did not seroconvert (OR=0.48; p=0.070). The MH test to determine if treatment allocation was an effect modifier in this relationship indicated there was a significant difference between the odds ratios stratified by treatment allocation (p=0.011) and the odds ratios should be reported separately. However, there were too few observations in sub-categories to calculate separate odds ratios. (No infants who shed RV but did not seroconvert in the vaccine arm and no infants who shed RV and seroconverted in placebo arm). Multivariate analysis could not be conducted as there were too few observations in sub-categories.

*a. ELISA Seroresponse rate six-months post-P2-VP8*

The association between the seroresponse rate of NAbs to RV strain 89-12 six months after receiving the third injection, adjusted for decay of maternal antibodies, and RV shedding was significant (p=0.020). Of 33 who shed RV, adjusted NAb seroresponse to RV strain 89-12 six

months after the third injection was shown in 28 (84.85%), compared with 69 (63.30%) of 109 who did not shed. Adjusted NAb response was significantly lower in those who did not shed (p=0.020). This relationship occurs in the opposite direction to the seroresponse one month after receiving the third injection. The unadjusted logistic regression indicated that individuals who had a positive NAb response to RV strain 89-12 were 3.79 times more likely to shed RV than individuals who did not have a positive seroresponse (OR=3.79; p=0.011). The MH test to determine if treatment allocation was an effect modifier in this relationship indicated there was no evidence of effect modification (p=0.589)

**Table 3.17: Final logistic regression model showing the relationship between NAb response to RV strain 89-12 six months after the third injection and RV shedding, as determined by ELISA (n=138).**

| Variables | AOR | Std. Err | z | P>\|z\| | 95% CI |
|---|---|---|---|---|---|
| mAb adjusted NAb to 89-12 seroresponse six-months post-P2-VP8 | | | | | |
| Positive | 4.08 | 2.28 | 2.51 | 0.012 | [1.36-12.23] |
| Treatment allocation | | | | | |
| Vaccine | 0.25 | 0.11 | -3.11 | 0.002 | [0.10-0.59] |
| Constant | 0.24 | 0.12 | -2.77 | 0.006 | [0.09-0.66] |

Table notes: NAb to RV strain Wa six-months post-P2-VP8 seroresponse is a categorical variable which is positive if the magnitude of the NAb antibody response to RV strain 89-12 measured six-months after receiving three doses of P2-VP8 vaccine or placebo is four-fold greater than the same response measured at baseline; NAb to RV strain 89-12 refers to the magnitude of the NAb antibody response to RV strain 89-12; mAb adjusted refers to titres which have been adjusted for the decay of maternal antibodies in infants as they get older; baseline refers to the analysis of sera conducted at baseline (enrolment); one-month post-P2-VP8 refers to the analysis of sera conducted one month after receiving three doses of parenteral P2-VP8-P[8] vaccine or placebo; six-months post-P2-VP8 refers to the analysis of sera conducted six months after receiving three doses of parenteral P2-VP8-P[8] vaccine or placebo; NAb: neutralising antibody; mAb: maternal antibody; AOR: adjusted odds ratio; Std. Err: standard error; CI: confidence interval. Treatment allocation, clinical haematology results (white blood cells, platelets, eosinophils, neutrophils, basophils, monocytes, lymphocytes), baseline IgG and IgA serum responses to P4, P6, and P[8], baseline IgA serum responses to whole RV lysate, and baseline NAb responses to RV strain Wa were all considered as covariates, treatment allocation was the only significant one (p=0.002). No confounders which modified the odds ratio by more than 10% were identified.

Table 3.17 shows the final adjusted logistic regression model, derived from backward selection, displaying the relationship between the NAb seroresponse to RV strain 89-12 six months post-P2-VP8 and RV shedding. Post-estimation tests indicate that this model was well specified and there was no evidence of lack of fit. A positive NAb seroresponse to RV strain 89-12 six months after receiving the third injection was associated with a 4.08-fold (95% CI: 1.36-12.23) increase in the odds of having shed RV in stools following Rotarix challenge (AOR= 4.08; 95% CI: [1.36-12.23]; p=0.012).

#### *3.2.5.3 Neutralising antibody to RV strain DS-1 and 1076*

Neutralising antibody responses to DS-1 and 1076 were low overall and there were no significant differences in GMTs or seroresponse between shedders and non-shedders at baseline or one-month post-P2-VP8 vaccination with P2-VP8-P[8] vaccine or placebo. Responses were not measured at the six-month post-P2-VP8 vaccination time point for DS-1 and 1076 strains.

## 3.3 ASSOCIATION BETWEEN POST-VACCINE P[8]-IGA IMMUNE RESPONSE ONE MONTH AFTER VACCINATION AND BASELINE IMMUNE RESPONSES

### 3.3.1 Association between P[8]-IgA seroresponse rate and baseline factors

#### *3.3.1.1 Bivariate analysis*

The association between P[8]-IgA seroresponse rate and baseline demographic factors and clinical immune responses was first investigated using bivariate tests (Table 3.18).

**Table 3.18: Association between P[8]-IgA seroresponse rate one-month post-P2-VP8 and baseline demographic and clinical immune response factors among P2-VP8-vaccine recipients.**

| Baseline clinical and demographic variables | IgA to P[8] seroresponse | | P-value |
|---|---|---|---|
| | Negative N=29 | Positive N=76 | |
| *Demographics* | | | |
| Sex, n(%) | | | 0.745 |
| Male | 14 (48.28) | 34 (70.83) | |
| Female | 15 (51.72) | 42 (55.26) | |
| Days of age, $\bar{x}$ [95% CI]† | 50.72 [49.70-51.77] | 50.68 [50.03-51.33] | 0.989 |
| *Haematology* | | | |
| White cell count (x10^9/L )† | 10.09 [9.29-10.96] | 9.73 [9.17 -10.33] | 0.630 |
| Hemoglobin (g/dL)† | 12.12 [11.58-12.68] | 11.67 [11.42-11.92] | **0.075** |
| Platelets (x10^9/L) | 482.92 [439.63-530.46] | 468.56 [437.94-501.32] | 0.792 |
| Neutrophil count (x10^9/L )† | 2.11 [1.85-2.40] | 2.15 [1.99-2.31] | 0.879 |
| Lymphocyte count (x10^9/L)† | 6.28 [5.72-6.90] | 5.91 [5.51-6.34] | 0.454 |
| Monocyte count (x10^9/L)† | 1.15 [0.97-1.36] | 1.10 [0.99-1.23] | 0.937 |
| Eosinophil count (x10^9/L)† | 0.27 [0.21-0.35] | 0.28 [0.24-0.34] | 0.820 |
| Basophil count (x10^9/L)†* | 0.02 [<0.01-0.04] | 0.06 [0.04-0.10] | 0.822 |
| *Antibodies to whole RV lysate* | | | |
| IgA to whole RV lysate GMT [95% CI] | 8.97 [7.30-11.01] | 9.13 [8.07-10.33] | 0.803 |
| *Antibodies to P2-VP8 sub-unit vaccine* | | | |
| IgA to P[8] GMT [95% CI] | 6.21 [4.71-8.18] | 5.68 [4.84-6.65] | 0.428 |
| IgG to P[8] GMT [95% CI] | 179.19 [111.74-287.33] | 104.98 [76.91-143.32] | **0.018** |
| *Neutralising antibodies to RV strains* | | | |
| NAb to Wa GMT [95% CI] | 98.08 [66.29-145.14] | 96.53 [74.80-124.58] | 0.805 |
| NAb to 1076† GMT [95% CI] | 44.93 [29.83-67.69] | 57.12 [43.29-75.37] | 0.203 |
| NAb to 8912 GMT [95% CI] | 133.51 [88.30-201.87] | 127.75 [98.50-165.67] | 0.766 |
| NAb to ds1† GMT [95% CI] | 75.21 [50.05-113.01] | 73.93 [56.65-96.46] | 0.906 |

Table notes: Pearson chi-squared tests were conducted for categorical variables, Wilcoxon Rank Sum was conducted for continuous variables unless variance was equal and a t-test was performed (indicated by a dagger†). *Asterisk indicates that arithmetic mean and not geometric mean was reported. Abbreviations: NAb: neutralising antibody, IgA: immunoglobulin A; IgG: immunoglobulin G; GMT: geometric mean titres; CI: confidence interval; n: number of observations.

Sex was not significantly associated with the P[8]-IgA seroresponse rate. Of the 48 males, 34 (70.83%) showed IgA serum anti-P2-VP8-P[8] seroresponse, compared with 42/57 (73.68%)

females (p=0.745). Age (days) was also not associated with the P[8]-IgA seroresponse rate. The mean age between positive and negative seroconverters were very similar with < 0.8 days difference (p=0.989).

Baseline haematology factors were not associated with the P[8]-IgA seroresponse rate. Haematology values between positive and negative IgA-P[8] seroconverters were not significantly different for white blood cells, haemoglobin density, platelets, neutrophils, lymphocytes, monocytes, eosinophils, or basophils (p>0.05 for all comparisons). Baseline IgA antibody titres to whole RV lysate were not associated with the P[8]-IgA seroresponse rate. The difference in geometric mean titres was < 1.00 and was not significantly different from 0 (p = 0.803). Baseline IgA titres to P[8], were not associated with P[8]-IgA seroresponse (P>0.05 for all comparisons). However, the magnitude of the baseline IgG response to P[8] was associated with the P[8]-IgA seroresponse (p=0.018). The difference in geometric mean titres between positive and negative seroconverters was 74.21. Individuals who seroconverted on the basis of P[8]-IgA had significantly lower titres of P[8]-IgG antibodies at baseline than individuals who do not seroconvert. There were no significant differences in baseline neutralising antibody titres to Wa, 8912, ds1 or1076 between seroconverters and non-seroconverters based on serum anti-P[8] IgA (p>0.05 for all comparisons).

#### *3.3.1.2 Multivariate analysis to determine effect of baseline immune responses on anti-P[8] IgA seroresponse*

The distribution of all variables, except for IgG antibodies to P[8], neutralising antibodies to RV strain Wa, and days of age, was not normally distributed. Log and cubic transformations were used to normalise the exceptions. A manual backward stepwise approach was used to determine which variables were associated with the P[8]-IgA seroresponse rate. All baseline clinical and demographic variables were included in the full model and then excluded one at a time. A likelihood ratio test was conducted after each variable was removed to test the null hypothesis that the more parsimonious model was the 'true' model. IgG to P[8] at baseline was found to be the only significant predictor at the 10% level of significance and Nab to Rv strain Wa at baseline was identified as a confounder which modified the odds ratio by more than 10%. No other predictors were found to be significant at the 5% level of significance. Keeping all other variables constant, for every 2.72 unit increase in the IgG antibody titres to P[8] at baseline, there is a 37% (95% CI: 0.41-0.98) decrease in the odds of seroconverting on the basis of P[8]-IgA (p=0.038;

Table 3.19). The postestimation tests indicated that there was no specification error.

**Table 3.19: Final model displaying association between IgG to P[8] and P[8] IgA seroresponse rate.**

| Variable | AOR | z | P-value | [95% CI] |
|---|---|---|---|---|
| IgG to P[8] at baseline (log titre) | 0.63 | -2.07 | 0.038 | [0.41-0.98] |
| NAb to Wa at baseline (log titre) | 1.34 | 1.16 | 0.246 | [0.82-2.19] |
| *Constant* | *6.71* | *1.79* | *0.073* | *[0.84-53.72]* |

Table notes: IgG to P[8] refers to the IgG antibody response to P2-VP8-P[8] vaccine antigen; NAb to Wa refers to the neutralising antibody response to RV strain; AOR: adjusted odds ratio; CI: confidence interval..

#### *3.3.1.3 Multivariate analysis to determine effect of baseline immune responses on anti-P[8] IgA magnitude*

A manual backward stepwise approach was used to determine which variables were associated with the magnitude of the P[8]-IgA response one month after receiving three doses of P2-VP8-P[8] vaccine. The magnitude of the P[8] IgA response was log-transformed to normalise the distribution. All baseline clinical and demographic variables were included in the full model and then excluded one at a time. A likelihood ratio test was conducted after each variable was removed to test the null hypothesis that the more parsimonious model was the 'true' model. The IgG response to P[8] was initially found to be the only significant predictor at the 10% level of significance, however, this was before including the NAb response to RV strain Wa at baseline which was a confounder that modified the odds ratio by more than 10%. After adjusting for this confounding effect there was no significant relationship between the IgG response to P[8] at baseline and the magnitude of the IgA response to P[8] one month after receiving the third injection.

# 4 CHAPTER FOUR: DISCUSSION

Correlates of protection are immune responses that are statistically associated with protection against clinical expression of disease. Immunogenicity trials for vaccines that assess correlates of protection require much smaller sample sizes than trials which assess clinical endpoints of the disease. Correlates of protection are beneficial for a number of reasons: to enable non-inferiority trials to support licensure of vaccines where placebo-controlled trials are no longer ethical (as in the case of RV where there is an existing albeit low-efficacy vaccine in some settings), to bridge the transition from first generation to second generation vaccines, to monitor the consistency of vaccine production, to study the susceptibility of a population after vaccination; and to enable the licensure of combination vaccines (Holmgren et al., 2017). For enteric vaccines, correlates of protection are currently associated with serological immune responses even though these may not be indicative of gut immune responses or protection (Holmgren et al., 2017). Several correlates of protection have been suggested for RV but none of these have been consistently understood or validated. Potential correlates of protection for live oral RV vaccines include serum IgA antibody detected by whole RV lysate ELISA, serum NAbs to RV strains (Gonzalez et al., 2004), RV-specific serum IgG antibodies, and shedding of RV in stools following vaccination (Holmgren et al., 2017).

Given that correlates of protection for RV are poorly understood and trials with clinical endpoints are expensive and difficult to conduct, the primary safety and immunogenicity trial of the monovalent P2-VP8-P[8] sub-unit vaccine, upon which this study is based, investigated whether reduction of RV faecal shedding following P2-VP8-P[8] vaccination could potentially be used as a correlate of protection (Groome et al., 2017b). Three doses of P2-VP8-P[8] vaccine or placebo were administered to infants followed by vaccination with Rotarix one month later. This was followed by ELISA-based detection of RV shedding in stools one week after the ORV challenge to identify whether infants were able to suppress local gut multiplication of the RV vaccine strain contained in Rotarix. Measures of immune responses to vaccination included serum IgA antibody responses to whole RV lysate, serum-binding antibodies to the P[8] vaccine antigen, and NAbs to P[8], P[6] and P[4] RV strains. Immune responses were measured at enrolment (baseline) and one (post-P2-VP8$^{1}$) and six months (post-P2-VP8$^{6}$) after receiving three doses of the P2-VP8-P[8] vaccine. The study found that RV shedding was significantly lower in infants who were vaccinated

with P2-VP8-P[8] compared to those who received the placebo, suggesting that the vaccine might induce immune responses at the gut surface which prevents virus multiplication (Groome et al., 2017b). However, further investigation into faecal shedding as a correlate of protection is required before it can be used as a surrogate marker of field efficacy. This secondary data analysis provided valuable insights into the use of faecal shedding following an ORV challenge as a measure of immune response to vaccination.

## 4.1 MEASURING RV SHEDDING IN STOOLS (PCR VERSUS ELISA)

It is important to establish the best way to measure shedding of the Rotarix vaccine strain in the stool after challenge. In the primary study, ELISA was used as the primary measure of shedding of rotavirus. ELISA has several advantages as it is a commercially-available test and is relatively cheap to do. Most commercial ELISA tests are sensitive and specific although this may vary between different tests (World Health Organization, 2009). A disadvantage of using ELISA for the purpose of Rotarix shedding is that it measures any rotavirus strain not just the Rotarix vaccine strain. In our study genotyping and sequencing of the ELISA-positive samples was conducted, and 9.09% of the infants who were shedding rotavirus were shedding a P[9] strain rather than the Rotarix strain. Including all ELISA-positive infants in the analysis, irrespective of the strain, would lead to a slight overestimation of Rotarix shedding and may have affected the results.

Quantitative PCR is a more sensitive, albeit more expensive, method of detecting rotavirus and PCR detecting VP6 antigen of Rotarix was also conducted on all the stool samples. This assay detects all strains of rotavirus and not just Rotarix-derived rotavirus strains. Using PCR increases the detection of rotavirus in the stool (Hsieh et al., 2014b) and a decision on the most appropriate ct-cut-off to define shedders versus non-shedders is needed. Using a more stringent cut-off (lower ct value) would increase specificity but decrease sensitivity, and using a less stringent cut-off (higher ct value) would increase sensitivity but decrease specificity. In our analysis, using an initial ct cut-off of 33, we found relatively poor correlation between the two testing methods. A ct cut-off of 21 had the best agreement. Our study highlights the need for further research into the best way to detect shedding of the Rotarix vaccine strain in stool samples after challenge, and how best to define shedders versus non-shedders. Additional analyses should be conducted using PCR at different ct cut-offs to define shedding, in the same way as we assessed associations between shedding via ELISA and serum immune responses.

## 4.2 INVESTIGATION INTO THE CORRELATION BETWEEN RV SHEDDING AND SERUM IMMUNE RESPONSES

As both RV faecal shedding and serum RV-specific immune responses have been proposed as potential correlates of protection for RV, this study investigated whether faecal shedding after Rotarix challenge and serum immune responses, observed one and six months after three doses of P2-VP8-P[8] vaccine, were correlated and thereby indicative of the same overall immune response to RV vaccination. We investigated associations between RV shedding in stools, as determined by ELISA, and post-P2-VP8 immune responses, adjusting for baseline covariates.

The primary study measured both IgA to whole RV lysate and IgA to the P2-VP8-P[8]vaccine antigen, P[8]. As IgA to whole RV lysate has been correlated with immune protection for live oral RV vaccines in some studies (Hjelt et al., 1987; Patel et al., 2013; Velázquez et al., 2000), albeit incompletely (Lee et al., 2018; Patel et al., 2013; Ward and Bernstein, 1995), this immune response has been used as a marker of immunogenicity for several RV vaccine trials (Araujo et al., 2007; Fix et al., 2015; Phua et al., 2005). However, it is likely that the correlate of protection for a protein-based non-replicating vaccine might be different, so serum-binding IgA antibodies to the vaccine antigen, P[8], were also measured in the primary study (Groome et al., 2017a).

Our secondary data analysis found that one month after P2-VP8 vaccination, the IgA immune response to the P[8] antigen, after adjusting for other covariates , was not significantly associated with RV shedding although both GMTs and seroresponses were ***higher in non-shedders*** than shedders. If reduced shedding of Rotarix is indicative of protection against rotavirus, then we would expect higher IgA immune responses in the non-shedders and lower IgA immune responses in the shedders. Overall, IgA seroresponse to whole RV lysate IgA was very low (7.48%) one-month post-P2-VP8 vaccination and was not significantly associated with RV faecal shedding.

Interestingly, six months after P2-VP8 vaccination, an inverse relationship was observed between IgA immune responses and RV shedding. IgA titres had increased considerably from baseline and IgA titres to both whole RV lysate and P[8] antigen were significantly ***higher in shedders*** compared to non-shedders, which was opposite to both the expected direction of difference and the general trend observed one month post-P2-VP8 vaccination. The adjusted odds ratio derived from stepwise logistic regression indicated that, keeping all other factors constant (including treatment allocation), those with high IgA titres six-months post-P2-VP8 were more likely to have shed RV following Rotarix challenge one month after receiving three doses of P2-VP8-P[8] vaccine or

placebo. This finding requires further investigation to properly understand the implications of what this means for RV shedding as a correlate of protection. Infants in both the vaccine and placebo groups would also all have received three doses of the ORV, Rotarix, by this time point. Our findings could indicate that those who shed RV one week after receiving their first dose of Rotarix, had a more robust immune response in the long term. As such, the P2-VP8-P[8] vaccine which resulted in reduced levels of shedding, may potentially have prevented the development of a robust IgA antibody response to whole RV lysate and P[8] in the long term. Alternatively, serum IgA at the later time point may have been measuring response to the ORV but may not have been a measure of immune response to the parenteral vaccine.

Several studies have indicated that serum IgG antibody is associated with protection against RV infection and illness (Clemens et al., 1992; Velazquez et al., 1996; Velázquez et al., 2000; Ward et al., 1989). As a result, our study also measured serum IgG response to the P[8] vaccine antigen. There was evidence of an association between IgG seroresponse to P[8], adjusted for maternal antibodies, and RV shedding one month post-P2-VP8 vaccination but there was no significant relationship between the magnitude of IgG titres and RV shedding. IgG antibody responses to P[8] six months after NRRV vaccination were not associated with RV shedding following Rotarix challenge in the bivariate analysis or in stepwise regression adjusting for other covariates. IgG antibody responses followed a similar pattern to that observed with IgA immune responses. IgG titres to P[8] were higher, albeit not significantly, in shedders compared to non-shedders one month-post-P2-VP8 vaccination and significantly higher in non-shedders compared to shedders six month post-P2-VP8 vaccination.

NAbs to RV strain target the VP4 and VP7 outer viral protein and prevent viral attachment and entry into cells (Ward, 2009; Ward et al., 2010). Intestinal NAb response to different RV strains have also been proposed as correlates of protection for RV (Ward, 2009; Ward et al., 2010; Wood and WHO Informal Consultative Group, 2005) but it is impractical to obtain small bowel secretions for measurement of intestinal antibodies (Grimwood et al., 1988). The correlation between serum NAb response to different RV strains and protection against RV infection seems to be context and strain specific(Angel et al., 2014). Some studies provide evidence to support serum NAbs as correlates of protection (O'Ryan et al., 1994; Ward et al., 1992) while others contradict this relationship (Ward et al., 1989; Zheng et al., 1989).

P[8]-type RV strains are responsible for the majority of RV infections worldwide, however P[6] and P[4] RV strains are important in South East Asia and Africa where they are responsible for approximately one third of RV infections. The primary study found that, following P2-VP8 vaccination, there was a strong response to P[8]-type RV strains in infants who had received the vaccination compared to infants who did not but this response was not exhibited for P[6] and P[4] type RV strains. This secondary analysis found that there was no association between RV shedding and NAb immune response to P[6]- and P[4]-type RV strains 1076 or DS-1 one month post-P2-VP8. In contrast, NAbs to P[8]-type RV strains, Wa and 89-12, were correlated with RV shedding following Rotarix vaccine challenge.

Initial bivariate analysis indicated that NAb titres to RV strain Wa one-month post-P2-VP8 were associated with RV shedding. This relationship was still significant following a stepwise logistic regression process. As with the other immune responses one-month post-P2-VP8, increased NAb titres to RV strain Wa were associated with a small, but significant, decrease in the odds of shedding RV following Rotarix challenge. This relationship indicates that infants with higher NAb to the RV strain, Wa, one month after P2-VP8 vaccination were less likely to shed RV following Rotarix vaccine challenge. The relationship between RV shedding and NAb responses six months post-P2-VP8 was also significant, and as demonstrated with IgA and IgG immune responses, this occurred in the opposite direction to the relationship observed at the one-month time-point. Those who had higher NAb titres to RV strain Wa six-month post-P2-VP8 vaccination were more likely to have shed RV following Rotarix challenge.

A similar pattern was observed for NAb response to RV strain 89-12. Titres of NAb to RV strain 89-12, one-month post-P2-VP8 vaccination, were higher in non-shedders than shedders. Six-months post-P2-VP8 vaccination, titres were significantly higher in shedders than non-shedders and the stepwise logistic regression model indicated that those with high titres were more likely to have shed RV following Rotarix vaccine challenge. Thus, infants were more likely to demonstrate a seroresponse against RV strain Wa and 89-12 if they shed RV after Rotarix challenge. This requires additional research but may indicate that the P2-VP8-P[8] vaccine may only have a temporary effect which may delay the development of immune responses to RV in the long-term.

Overall non-shedders had higher immune responses one-month post-P2-VP8 than shedders and seroresponse was more frequent amongst non-shedders than shedders. While the association between the serum rotavirus-specific immune response and RV shedding was not always

statistically significant, there was a general trend towards higher immune responses in non-shedders compared to those shedding RV post challenge. Infants who had higher serum immune responses were less likely to shed RV after the Rotarix challenge dose. If reduced RV shedding is indicative of a local gut immune response and suppression of replication of the Rotarix vaccine strain, then we would expect serum rotavirus immune responses to be higher among non-shedders. The non-significance of some of the results may merely have been due to the sample size. Additional investigations, using an increased sample size, are needed to further assess these relationships.

Conversely, six months post-P2-VP8 vaccination, RV shedders had higher immune responses than non-shedders, and IgA serum antibodies to the P[8] vaccine antigen and whole RV lysate, and NAbs to P[8]-type RV strains Wa and 89-12 were significantly associated with RV shedding following Rotarix challenge. Those with higher immune responses (IgA anti-P[8], IgA anti-whole RV lysate, NAb to RV strain Wa and 89-12) six months post P2-VP8 vaccination were more likely to have shed RV following Rotarix challenge. This could possibly reflect the fact that RV shedding after ORV challenge is indicative of the "vaccine take" of the first Rotarix dose, and is predictive of higher serum immune responses at this later time point. The relationships we see between RV shedding and serum immune responses at nine months of age may also possibly be influenced by the same factors that have been hypothesised to be responsible for the lower efficacy and immunogenicity observed in the clinical trials assessing ORVs(Velasquez et al., 2017b). Factors such as enteric co-infections at the time of ORV vaccination, breast milk antibodies and host genetic factors such as HBGAs may affect RV shedding after the challenge dose, and thus confound the relationship between shedding and serum immune responses. In order to adequately assess the Rotarix challenge model and the measure of RV shedding post challenge, future evaluations need to take these factors into account. A breastfeeding history, stool analysis for enteric infections at the time of the challenge dose and collection of saliva or DNA to determine infant Lewis and secretor would be important additions in future studies. An alternative reason for the higher antibody response six-months post-P2-VP8 is that the parenteral P2-VP8-P[8] sub-unit vaccine may not be as efficacious as the oral vaccine.

## 4.3 Association between IgA to P[8] antigen and baseline factors

We also investigated whether baseline immune responses affected IgA titres and seroresponse to the P[8] vaccine antigen. We hypothesised that presence of pre-existing immune responses as a

result of prior exposure to RV or RV antigens would be associated with improved IgA seroresponse to P[8] and higher anti-P[8] IgA titres one-month after receiving three doses of the P2-VP8-P[8] vaccine. This would support an argument for pursuing a prime-boost strategy i.e. priming with ORV and boosting with a parenteral vaccine. Bivariate tests were conducted to identify whether anti-P[8] IgA seroresponse was associated with baseline immune responses in vaccine recipients only. Immune responses that were investigated included baseline serum binding IgG and IgA antibodies to P[8], IgA antibody titres to whole RV lysate, and NAb titres to RV strains 89-12, DS1, 1076, and Wa, in addition to clinical haematology results. Multivariate analysis was also conducted using both anti-P[8] IgA seroresponse and magnitude as dichotomous and continuous outcome variables, respectively.

This analysis found that neither baseline serum binding IgA antibody titres to whole RV lysate, P[8] nor NAb titres to RV strains 89-12, DS1, 1076, and Wa were associated with either IgA titres or IgA antibody seroresponse to P[8] one month post-P2-VP8 vaccination. This was observed in both the bivariate and multivariate analyses. Baseline serum binding IgG antibody titres to P[8], however, were found to be associated with anti-P[8] IgA seroresponse. Both bivariate and multivariate analyses indicated that lower IgG titres to P[8] at baseline were associated with positive anti-P[8] IgA seroresponse. This indicated that those with high IgG titres to P[8] were less likely to seroconvert one month post-P2-VP8 vaccination. This relationship occurs in the opposite direction to what is expected as evidence for prime boost. It is likely that infants have higher IgG titres to P[8] at baseline as a result of the presence of maternal antibodies. These maternal antibodies could mask the vaccine antigen preventing the development of an immune response to P2-VP8-P[8] as shown in other studies(Groome et al., 2014a; Nguyen et al., 2006; Yang et al., 2019). Thus, a parenteral rotavirus vaccine may also be subject to interference from maternal antibodies transferred to the infants.

While further research is required, the results of this preliminary analysis suggest that being primed and having higher baseline immune responses were not associated with increased seroresponse or antibody titres to P[8] one month after receiving three doses of P2-VP8-P[8] vaccine. This suggests that prime-boost strategies may not be indicated for P2-VP8-P[8]. However, this lack of association could also be due to the fact that serum binding IgA antibodies to P[8] may not be an effective correlate of protection for RV. If this is not an appropriate correlate, then it is likely that the

incorrect immune responses are being measured. Further analysis is required to identify a specific correlate of protection for RV before this analysis can be successfully concluded.

## 4.4 LIMITATIONS

This secondary analysis has several limitations which need to be considered before drawing any conclusions from the results. Firstly, the study was powered for safety and immunogenicity endpoints for the primary study, and not for this secondary analysis. The lack of significance may have been due to the sample size available for this analysis and a larger sample size may be needed to show significant differences in serum immune responses one-month post vaccination between shedders and non-shedders. Due to the limited sample size it was not also possible to conduct the separate analyses for vaccine recipients and placebos. Selection of variables for the multivariate logistic regression models were performed using a manual backward stepwise approach, but it is possible that this approach could include variables that are not necessary. Important confounders such as Lewis and secretor status, enteric co-infections and breastfeeding were not measured in the primary study. The analyses could therefore not adjust for these factors. In addition to receipt of three doses of Rotarix, we know that there was circulation of wild-type RV during the study period as evidenced by the sequencing of the ELISA-positive samples – 9.09% were shedding a G9 strain rather than the Rotarix strain. Exposure to wild-type virus may thus also have confounded our results and complicated the interpretation.

# 5 CHAPTER FIVE: CONCLUSION

Correlates of protection are important measures in vaccine development which enable the rapid and cost-effective development of new vaccines for diseases, such as RV, where licenced vaccines have low effectiveness, especially in LMICs. While there is some research documenting potential correlates of protection, such as RV-specific serum IgA and IgG, and NAbs to RV-strains; overall, correlates of protection for RV have not been consistently identified. Shedding of RV following ORV challenge has been investigated as a potential model to assess protection of parenteral vaccines administered prior to ORV challenge but this needs further evaluation before this can be used as a measure of field efficacy in RV vaccine trials.

Shedding of RV in faecal samples can be measured via PCR and ELISA; both methods were used in the primary study. There was poor agreement between PCR and ELISA measurements when using a ct cut-off of 33, and further research is required to identify the appropriate ct cut-off that can be used to distinguish shedders from non-shedders. ELISA was used as the primary method of detection in this secondary analysis due to its commercial availability and wide-applicability.

This study investigated whether RV shedding following Rotarix challenge was associated with other immune responses after receiving three doses of P2-VP8-P[8] vaccine or placebo. As expected, this study found that serum RV-specific immune responses measured *one*-month post-P2-VP8 vaccination tended to be higher in infants that did not shed RV post-challenge than infants who shed. The associations were not all statistically significant but the trends were in the direction we expected. Lack of significance observed could be a result of a limited sample size and additional investigations with increased sample size are required.

In contrast, RV shedding was highly correlated with immune responses *six* months post-P2-VP8 vaccination and the relationship at the six-month time point was in the opposite direction to what was expected. Those who had higher serum RV-specific immune responses were significantly more likely to have shed RV in stools after the first dose of Rotarix was administered compared to those who had lower serum RV-specific immune responses. This suggests that RV shedding may be reflective of vaccine take following ORV as shedders had significantly higher immune responses six-months post-vaccination.

In the absence of an absolute immunological correlate of protection for RV, it is very difficult to establish the usefulness of this faecal shedding model using only immunological correlations. In order to clearly establish whether shedding post ORV challenge can be used as a marker of field efficacy, further studies are needed to investigate whether faecal shedding post ORV challenge is correlated with clinical protection. In addition, any evaluation of shedding needs to take other potential confounders into account, including Lewis secretor status, breastmilk antibodies, and presence of enteric co-infections at the time of vaccination.

This study also investigated whether there was an association between baseline immune responses and IgA titers and seroresponse to the P[8] vaccine antigen one-month post-vaccination. If there was an association between baseline and post-vaccination immune response, this might indicate that a prime-boost strategy would be effective for the P2-VP8-P[8] vaccine. Associations between baseline immune responses and IgA responses to the P[8] vaccine antigen one-month post-vaccination were not statistically significant. However, to determine whether a prime-boost strategy is indicated, IgA seroresponse to the P[8] vaccine antigen needs to be validated as a correlate of protection. The lack of association could either indicate that a prime-boost strategy is not indicated, or that the incorrect immune responses are being measured. Further research is required before this question can be answered.

Given the limitations of this secondary analysis such as small sample size for sub-categories, and inability to account for all potential confounders, it is clear that additional research is required before the study questions can be adequately answered. Our results have demonstrated that there is a need for more research into both the immunogenicity of the P2-VP8 vaccine and correlates of protection for RV with a specific focus on the RV shedding following ORV challenge.

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# APPENDICES

## A. PLAGIARISM DECLARATION

I Tamika Fellows (Student number: 672041) am a student registered for the degree of Master of Science in Biostatistics and Epidemiology in the academic year 2020.

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
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- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.

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https://espace.library.uq.edu.au/data/UQ_138c56b/s4282820_final_thesis.pdf?Expires=1551580712&Key-Pair-Id=APKAJKNBJ4MJBJNC6NLQ&Signature=Qi~GgIGtcO1oVkw0Z0DeQIc2~anrfhZrKJQ300DPZt9PRQqgvpqNhGR~Kkq34NLQ6UQJ8IPcaTGIi6HRIg4NZMZBg0IT0blue9NrP2PLOFnNhbTk-bgj8WFNkduC0r5LmAF2A7YCaInmdORJDBKKQ2xJbQsIWcF8cX3pSPVcHszYqtanyDEDDq4JgVB6SnRI09Ox8R23UXXFwz5FCK-RI6DjMaaWgzhFXpquglit4y7r~oJCp9vbESwtHvySP0Hm-awsIjGTG8xDnwO5kVGVY8PSwR8EXeTE0ow8XOov6j7n4xgCIrQIQ__

< 1% match (Internet from 18-Oct-2017)
http://discovery.ucl.ac.uk/1389019/2/Risk_for_Eating_Disorders_and_Neuropsychological_Functioning_Final_redacted_version.pdf, kothari.p

< 1% match (Internet from 12-Apr-2010)
http://malariajournal.com/content/pdf/1475-2875-7-142.pdf

< 1% match (Internet from 12-Apr-2020)
https://worldwidescience.org/topicpages/i/injury+severity+scores.html

< 1% match (publications)
"Handbook of Disease Burdens and Quality of Life Measures", Springer Nature, 2010

< 1% match (publications)
Anthonet Koen, Lisa Jose, Shabir A. Madhi, Alan Fix, Stanley Cryz, Michelle J. Groome, "Neutrophil Counts in Healthy South African Infants: Implications for Enrollment and Adverse Event Grading in Clinical Trials in an African Setting", The Journal of Pediatrics: X, 2019

< 1% match (publications)
Jan Holmgren, Umesh D. Parashar, Stanley Plotkin, Jacques Louis et al. "Correlates of protection for enteric vaccines", Vaccine, 2017

< 1% match (Internet from 07-Feb-2020)
https://www.hse.ie/eng/services/publications/clinical-strategy-and-programmes/national-laboratory-handbook-volume-1.pdf

< 1% match (Internet from 03-Jun-2012)
http://www.incubationsystems.com/pdf_chapter_one_introduction.html

< 1% match (publications)
Marieke L. A. de Hoog, Timo Vesikari, Carlo Giaquinto, Hans-Iko Huppertz, Federico Martinon-Torres, Patricia Bruijning-Verhagen, "Report of the 5th European expert meeting on rotavirus vaccination (EEROVAC)", Human Vaccines & Immunotherapeutics, 2018

< 1% match (Internet from 18-Mar-2020)

## C. ETHICS CLEARANCE CERTIFICATE

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R14/49 Miss Tameka Fellows

**HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)**

**CLEARANCE CERTIFICATE NO. M181002**

| | |
|---|---|
| **NAME: (Principal Investigator)** | Miss Tameka Fellows |
| **DEPARTMENT:** | Public Health<br>Respiratory Meningeal Pathogens Research Unit |
| **PROJECT TITLE:** | Investigating factors affecting responses to rotavirus vaccination in a randomised control trial in South Africa |
| **DATE CONSIDERED:** | 26/10/2018 |
| **DECISION:** | Approved unconditionally |
| **CONDITIONS:** | |
| **SUPERVISOR:** | Dr Michelle Groome |
| **APPROVED BY:** | Doctor CB Penny, Chairperson, HREC (Medical) |
| **DATE OF APPROVAL:** | 05/12/2018 |

**This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.**

**DECLARATION OF INVESTIGATORS**

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in October and will therefore be due in the month of October each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

20 January 2018
Date

## D. STUDY INCLUSION AND EXCLUSION CRITERIA

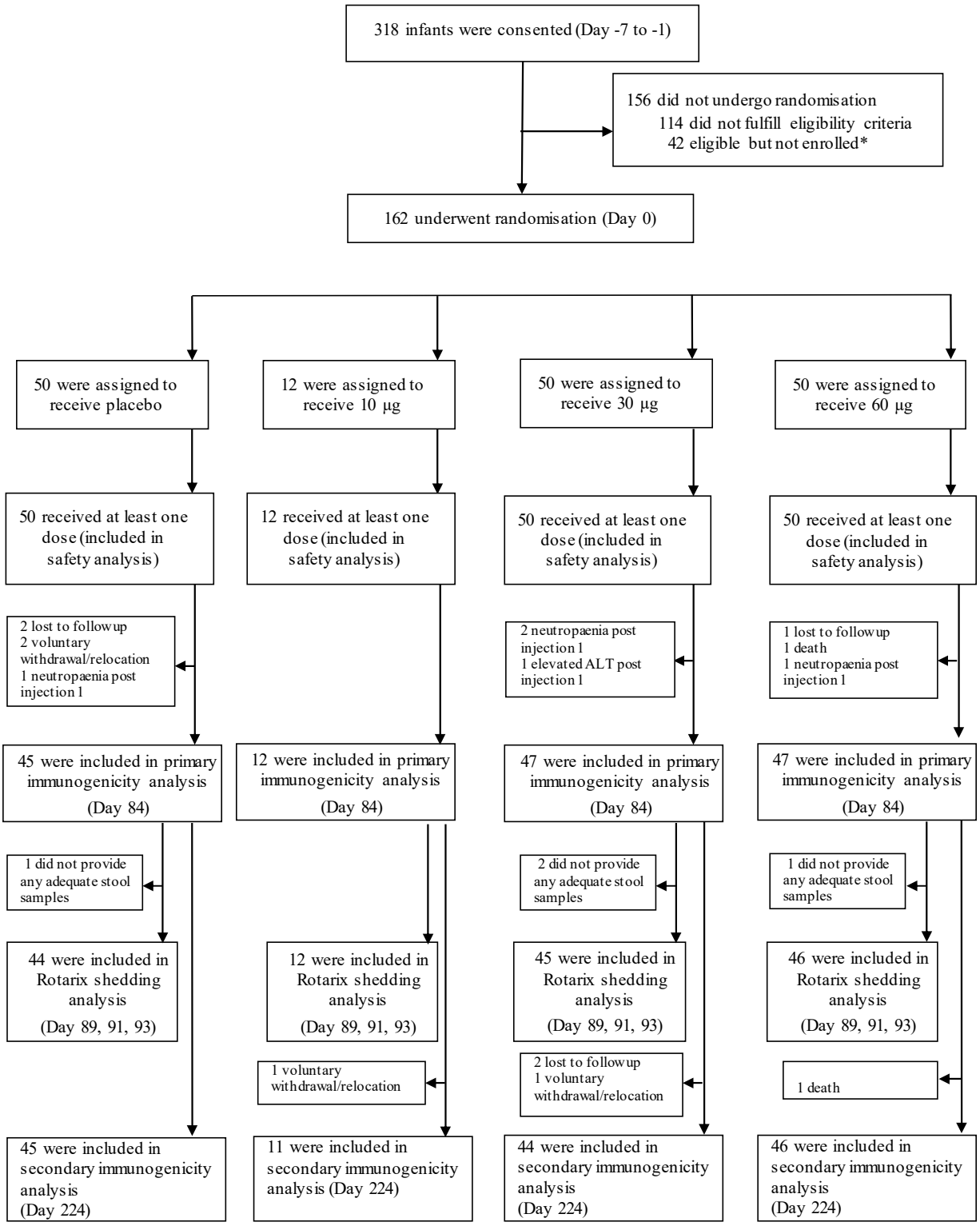
### Inclusion criteria

1. Healthy infants/toddlers as established by medical history and clinical examination before entering the study
2. Age of infant cohort: ≥6 and <8 weeks at the time of enrollment
3. Parental ability and willingness to provide informed consent
4. Parental intention to remain in the area with the child during the study period

### Exclusion Criteria

1. Presence of fever on the day of enrollment
2. Acute disease at the time of enrollment
3. Concurrent participation in another clinical trial throughout the entire timeframe for this study
4. Presence of malnutrition or any systemic disorder (cardiovascular, pulmonary, hepatic, renal, gastrointestinal, hematological, endocrine, immunological, dermatological, neurological, cancer or autoimmune disease) as determined by medical history and/or physical examination that would compromise the participant's health or is likely to result in nonconformance to the protocol
5. For infant cohort, history of premature birth (<37 weeks gestation)
6. History of congenital abdominal disorders, intussusception, or abdominal surgery
7. Known or suspected impairment of immunological function based on medical history and physical examination
8. For infant cohort only, prior receipt of rotavirus vaccine
9. A known sensitivity or allergy to any components of the study vaccine
10. History of anaphylactic reaction
11. Major congenital or genetic defect
12. Participant's parents not able, available or willing to accept active weekly follow-up by the study staff
13. Has received any immunoglobulin therapy and/or blood products since birth or planned administration during the study period
14. History of chronic administration (defined as more than 14 days) of immunosuppressant medications, including corticosteroids. Infants on inhaled or topical steroids may be permitted to participate in the study
15. Any medical condition in the parents/infant that, in the judgment of the investigator, would interfere with or serves as a contraindication to protocol adherence or a participant's parents' ability to give informed consent
16. HIV infection
    a. For toddlers, to be assessed by HIV ELISA
    b. For infants, to be assessed by PCR, if mother is not known to be negative (negative test result between 24 weeks gestation and screening)

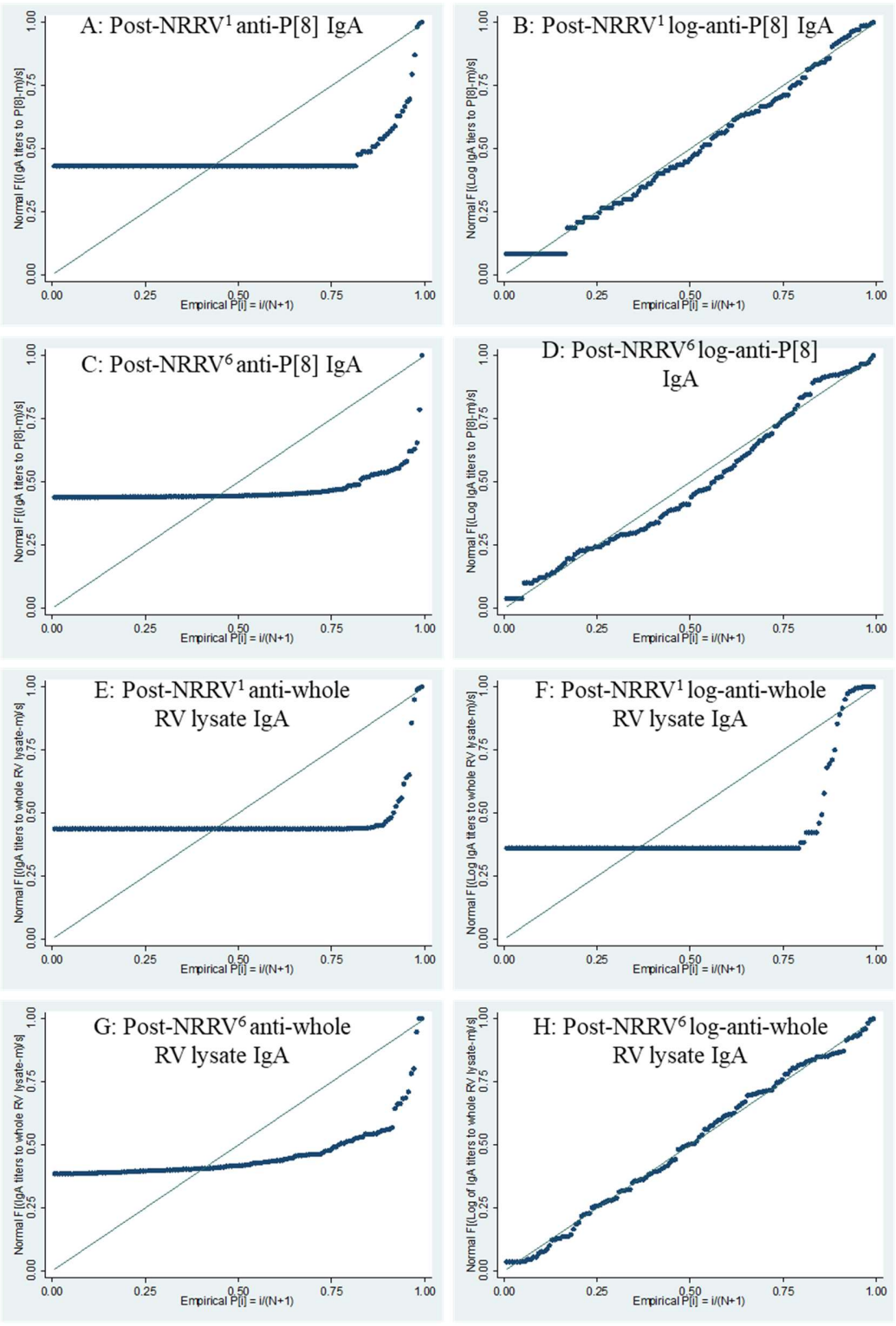
## E. PRIMARY STUDY SAMPLE

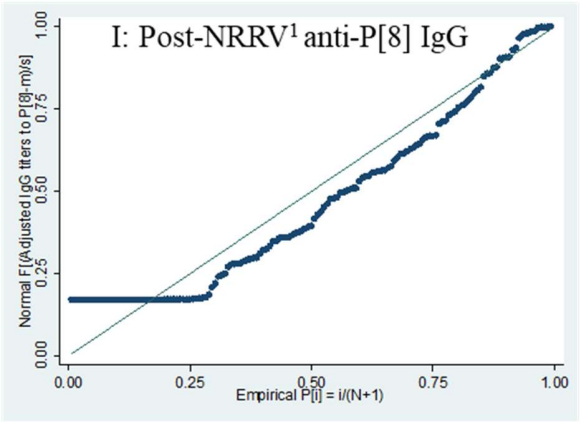


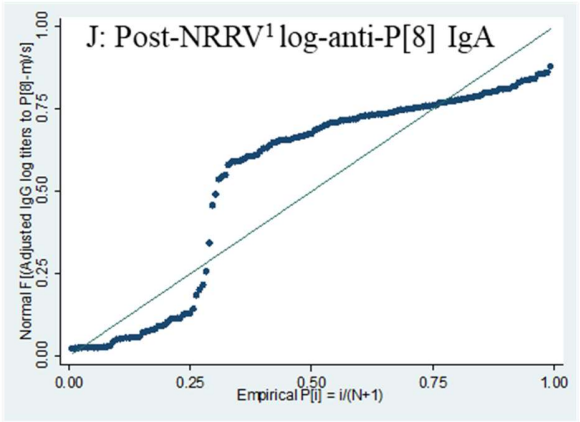

*16 enrolment postponed or enrolment into dosing group complete, 16 no longer interested, 7 unsuccessful phlebotomy, 3 other

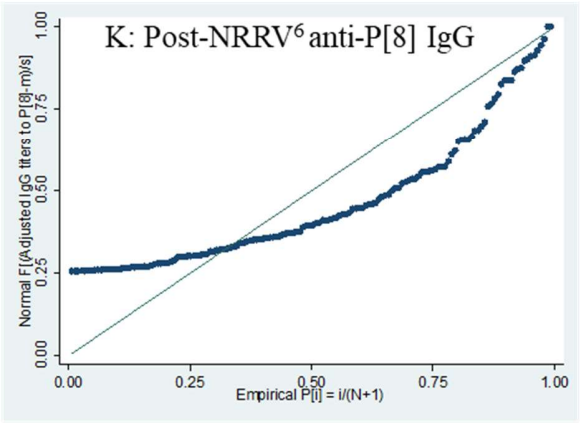
## F. STANDARDISED NORMAL PROBABILITY PLOTS

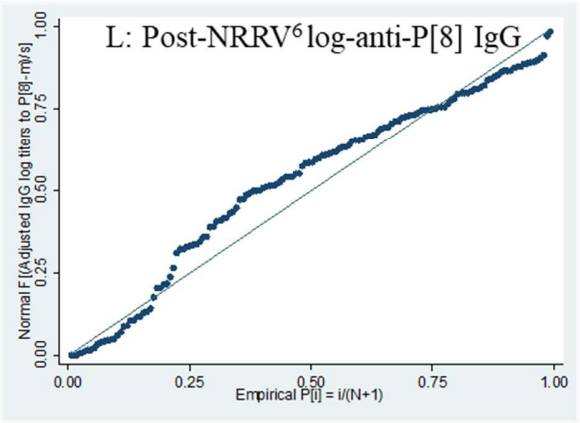
Standardised normal probability plot of untransformed and log-transformed titres one- and six-months after receiving three doses of P2-VP8-P[8] vaccine or placebo.

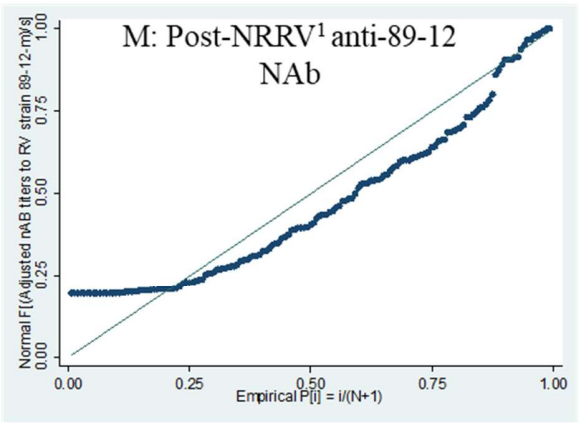


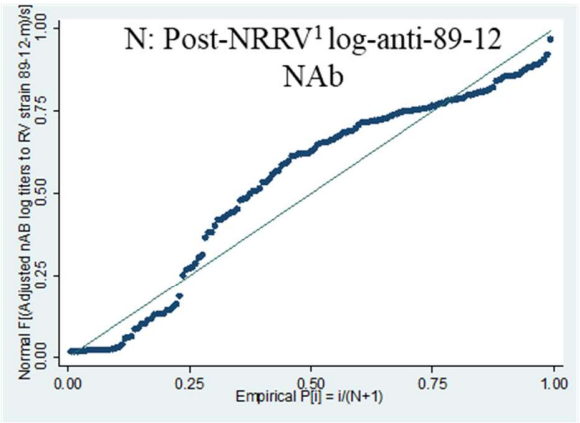

I: Post-NRRV1 anti-P[8] IgG
Normal F[(Adjusted IgG titers to P[8]-m)/s]
Empirical P[i] = i/(N+1)
0.00
0.25
0.50
0.75
1.00

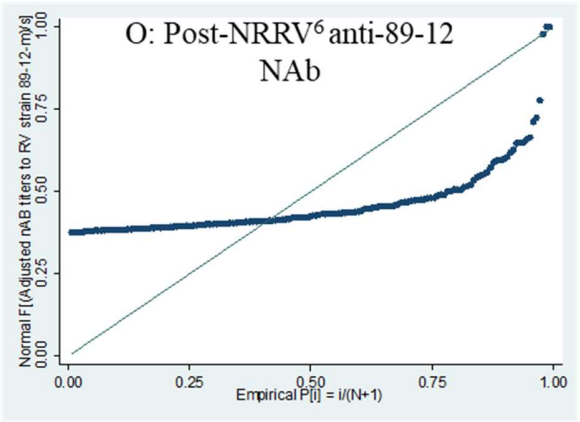

J: Post-NRRV1 log-anti-P[8] IgA
Normal F[(Adjusted IgG log titers to P[8]-m)/s]
Empirical P[i] = i/(N+1)
0.00
0.25
0.50
0.75
1.00

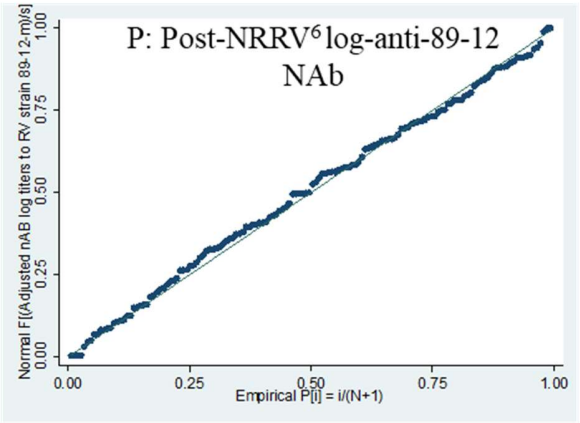

K: Post-NRRV6 anti-P[8] IgG
Normal F[(Adjusted IgG titers to P[8]-m)/s]
Empirical P[i] = i/(N+1)
0.00
0.25
0.50
0.75
1.00

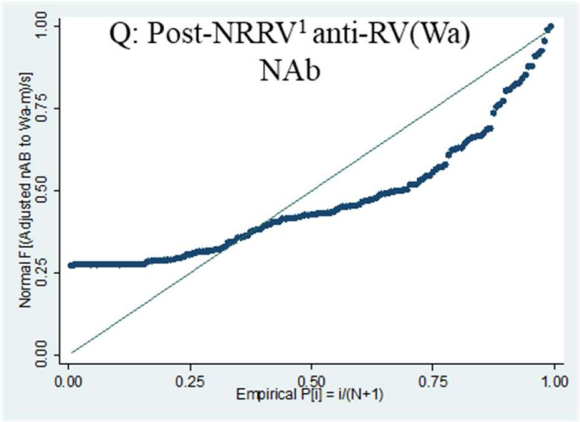

L: Post-NRRV6 log-anti-P[8] IgG
Normal F[(Adjusted IgG log titers to P[8]-m)/s]
Empirical P[i] = i/(N+1)
0.00
0.25
0.50
0.75
1.00

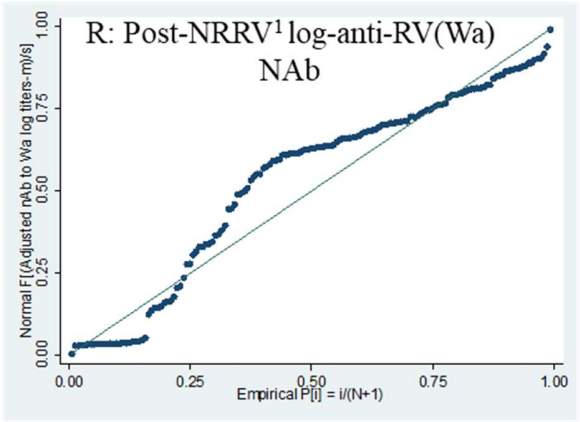

M: Post-NRRV1 anti-89-12 NAb
Normal F[(Adjusted nAB titers to RV strain 89-12-m)/s]
Empirical P[i] = i/(N+1)
0.00
0.25
0.50
0.75
1.00

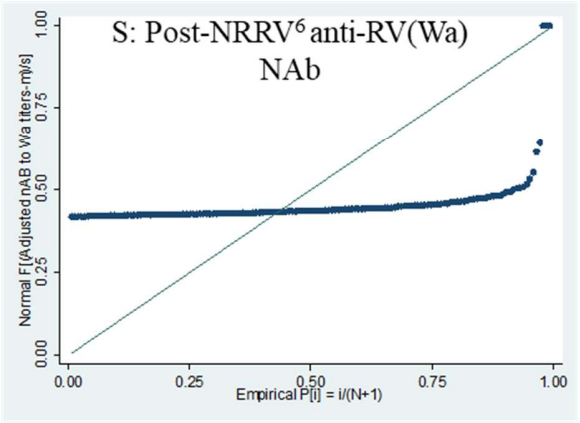

N: Post-NRRV1 log-anti-89-12 NAb
Normal F[(Adjusted nAB log titers to RV strain 89-12-m)/s]
Empirical P[i] = i/(N+1)
0.00
0.25
0.50
0.75
1.00

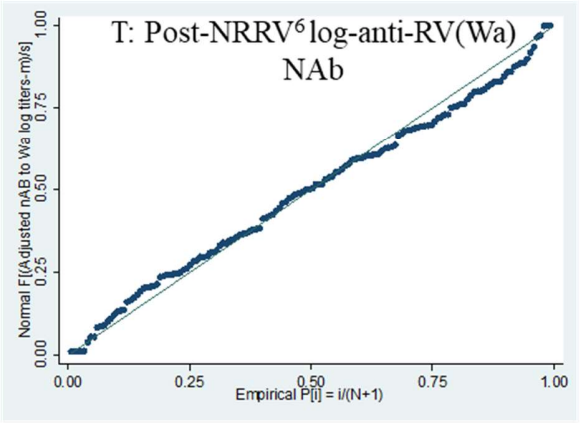

O: Post-NRRV6 anti-89-12 NAb
Normal F[(Adjusted nAB titers to RV strain 89-12-m)/s]
Empirical P[i] = i/(N+1)
0.00
0.25
0.50
0.75
1.00


P: Post-NRRV6 log-anti-89-12 NAb
Normal F[(Adjusted nAB log titers to RV strain 89-12-m)/s]
Empirical P[i] = i/(N+1)
0.00
0.25
0.50
0.75
1.00


Q: Post-NRRV1 anti-RV(Wa) NAb
Normal F[(Adjusted nAB to Wa-m)/s]
Empirical P[i] = i/(N+1)
0.00
0.25
0.50
0.75
1.00


R: Post-NRRV1 log-anti-RV(Wa) NAb
Normal F[(Adjusted nAb to Wa log titers-m)/s]
Empirical P[i] = i/(N+1)
0.00
0.25
0.50
0.75
1.00


S: Post-NRRV6 anti-RV(Wa) NAb
Normal F[(Adjusted nAB to Wa titers-m)/s]
Empirical P[i] = i/(N+1)
0.00
0.25
0.50
0.75
1.00


T: Post-NRRV6 log-anti-RV(Wa) NAb
Normal F[(Adjusted nAB to Wa log titers-m)/s]
Empirical P[i] = i/(N+1)
0.00
0.25
0.50
0.75
1.00

## G. Odds ratios for Final Adjusted Models

