The role of the intestinal microbiome in rotavirus vaccine immunogenicity
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Chapter One

General Introduction
General Introduction

Rotavirus disease

Over the past 5 decades anywhere on earth, the risk of a child dying before they reach their 5th birthday has decreased dramatically (Wang et al., 2017). Despite this monumental public health achievement, the global under-five mortality burden is borne unequally across countries. In 2016 only 3.8 children per 1000 livebirths died in the Netherlands before reaching the age of 4 whereas in Ghana this number was 52.3, in Pakistan 53.4 and in the Central African Republic as high as 130.5 deaths (Wang et al., 2017). After the first month of life, the predominant diseases causing these childhood deaths are diarrhea and pneumonia, collectively responsible for up to 28% of under-5 deaths globally (Black et al., 2010).

Global health research has made it possible to describe in increasing detail which infectious diseases are driving childhood pneumonia and diarrheal mortality in developing countries (Walker et al., 2013). Rotavirus is the most common cause of severe and life-threatening gastroenteritis in young children in developing countries (Platts-Mills et al., 2016). Prior to rotavirus vaccine introduction, diarrhea attributable to rotavirus caused an estimated 453,000 deaths worldwide (37% of diarrheal deaths and 5% of all deaths) in children under five (Tate et al., 2012). By 2013, this number was still 215,000 per year and the vast majority of these deaths were in sub Saharan African and Asia (Tate et al., 2016) (Figure 1).

Figure 1: Rotavirus-associated mortality in children <5 years of age in 2013. As of April 2016, the WHO estimated that worldwide, 215,000 children < 5 years of age died because of rotavirus infection in 2013. (Figure reprinted with permission from the World Health Organization. http://www.who.int/immunization/monitoring_surveillance/burden/estimates/rotavirus/rotavirus_deaths_map_b.jpg?ue=1. Accessed February 28, 2018.)
Rotavirus pathophysiology

Rotavirus is a double-stranded RNA virus of the family Reoviridae that infects enterocytes and enteroendocrine cells such as enterochromaffin cells in the small intestine (Saxena et al., 2015). The virus is a sphere with outer spikes and derives its name from the Latin root rota, or wheel. (Figure 2) In the sphere, three concentric layers surround 11 segments of dsRNA. The RNA segments encode six structural viral proteins (VP1, VP2, VP3, VP4, VP6 and VP7) and six non-structural proteins (NSP1, NSP2, NSP3, NSP4, NSP5 and NSP6). The structural proteins determine the architecture of the virus: the inner capsid and virion core are made up of VP2 with the enzymes VP1 and VP3; the middle capsid is VP6, which determines host specificity; the outer capsid displays the epitopes that elicit host immune responses - VP7, a smooth protein, and VP4, a protruding spike protein that is cleaved into VP8* and VP5* (Figure 2). The non-structural proteins are needed for rotavirus replication, contain the enterotoxin NSP4, and can antagonize host innate immune responses (via NSP1)(Crawford et al., 2017).

Rotavirus causes particularly severe gastroenteritis in young children. Transmitted by fecal-oral contamination, the virus transits to the small intestine and enters host enterocytes through binding of the outer spike protein, VP4, to sialoglycans.

Figure 2: Schematic representation of a rotavirus virion. The virus is composed of three protein shells, an outer capsid, an inner capsid, and an internal core, that surround the 11 segments of double-stranded RNA. The outer capsid proteins VP4 and VP7 are neutralization antigens and define the P and G serotypes, respectively. VP6, the inner capsid structural protein, is the subgroup antigen. (Reprinted with permission from J. Angel, M.A. Franco, and H.B. Greenberg. 2007. Rotavirus vaccines: recent developments and future considerations. Nat. Rev. Microbiol. 5:529–539.)
and perhaps histo-blood group antigens (HBGA) in a strain-dependent manner (Arias et al., 2015). The virus is then internalized by endocytosis.

Clinical manifestations of rotavirus disease include diarrhea, vomiting, and fever. The diarrhea is primarily secretory through increased intracellular calcium up-regulating calcium-dependent chloride channels and chloride secretion following NSP4 enterotoxin signaling (Ball et al., 1996; Ko et al., 2014). Malabsorption secondary to enterocyte damage (Crawford et al., 2017) and increased intestinal motility due to serotonin release from enterochromaffin cells also play a role in diarrhea (Bialowas et al., 2016). Vomiting resulting in dehydration, an important clinical manifestation that exacerbates the severity of rotavirus disease, is caused by NSP4-induced serotonin release activating vagal afferent nerves (Hagbom et al., 2011).

Immunity to rotavirus is incompletely understood partially due to differences in in vitro and animal findings and clinical studies. Rotavirus is able to evade the innate immune system. Rotavirus is recognized by several pattern recognition receptors (PRRs) but it has evolved mechanisms to inhibit PRR signaling through NSP1 interference (Arnold et al., 2013). Adults and children are subject to recurrent infections (Gladstone et al., 2011; Ward et al., 1989) suggesting that adaptive immunity to RV is poor, potentially a result of NSP1 alteration of innate immunity. Currently, serum anti RV IgA is the best correlate of protection for natural and vaccine-induced immunity (Angel et al., 2012; Patel et al., 2013) but is not a perfect correlate. Both antibodies and T cells are likely important in protection against disease, as infants with SCID develop chronic rotavirus infection (Klinkenberg et al., 2015).

**Rotavirus vaccines**

In 1998, Rotashield was the first licensed vaccine to prevent severe rotavirus gastroenteritis in infants. The vaccine was withdrawn from the market as a result of post-licensure reporting of an increased risk of intussusception. It took till 2006 for Merck to license RotaTeq (RV5), a three-dose pentavalent, bovine re-assortant live oral rotavirus vaccine, and for Glaxosmithkline to license Rotarix (RV1), a two-dose live attenuated G1[P8] human strain vaccine. These vaccines have been broadly introduced in the US and countries in Europe, South America, and Africa and Asia, often with GAVI support. India approved licensure of an inexpensive human-bovine monovalent rotavirus vaccine, ROTAVAC, in 2016 for national use, and several other rotavirus vaccines are licensed or in the development pipeline (Kirkwood et al., 2017). (Table 1) Countries in Asia and the Western Pacific
have been particularly slow to introduce rotavirus vaccines and currently 67% of children globally still lack access to rotavirus vaccines (International Vaccine Access Center (IVAC)).

Although many children are not protected against severe rotavirus gastroenteritis because they are not getting vaccinated, the currently licensed RV vaccines also have significantly impaired protection when administered in developing countries as compared to developed countries (Fischer Walker and Black, 2011). This characteristic is shared with several other orally administered vaccines, including live-attenuated and heat-killed vaccines against viral and bacterial pathogens such as polio, cholera, shigella, and typhoid (Qadri et al., 2013). Prior to deliberating on a global recommendation for rotavirus vaccine introduction, the WHO requested data on RV1 and RV5 vaccine efficacy in developing country settings. While trials of RV1 and RV5 demonstrated very high RVV efficacy rates against severe gastroenteritis in developed countries – 85% and 98%, respectively, data from developing countries was much lower (Ruiz-Palacios et al., 2006; Vesikari et al., 2006). RV1 trials in Malawi and South Africa demonstrated a pooled vaccine efficacy of 61.2% against severe RV gastroenteritis in the first year of life (Madhi et al., 2010) with waning immunity in the second year of life. A parallel RV5 trial showed a vaccine efficacy of 39.3% in Ghana, Kenya and Mali (Armah et al., 2010) and 48.3% in Bangladesh and Vietnam (Zaman et al., 2010). ROTAVAC (introduced later using a neonatal reassortant G9P[11] strain) had an efficacy of 53.6% in

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### Table 1: Overview of currently licensed vaccines with manufacturer and strain

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Manufacturer</th>
<th>Strain</th>
<th>Licensed</th>
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<tbody>
<tr>
<td>RotaTeq&lt;sup&gt;R&lt;/sup&gt;</td>
<td>Merck &amp; Co, USA</td>
<td>a pentavalent human-bovine reassortant vaccine</td>
<td>Globally, WHO pre-approved</td>
</tr>
<tr>
<td>Rotarix&lt;sup&gt;TM&lt;/sup&gt;</td>
<td>GlaxoSmithKline Biologicals, Belgium</td>
<td>a human monovalent (G1P[8])</td>
<td>Globally, WHO pre-approved</td>
</tr>
<tr>
<td>ROTAVAC</td>
<td>Bharat Pharmaceuticals, India</td>
<td>116E, naturally-occurring human reassortant strain (G9P[11])</td>
<td>India</td>
</tr>
<tr>
<td>Rotavin-M1</td>
<td>The Center for Research and Production of Vaccines and Biologicals (POLYVAC), Vietnam</td>
<td>KH0118-2003, naturally-occurring attenuated human strain (G1P[8])</td>
<td>Vietnam</td>
</tr>
<tr>
<td>Lanzhou Lamb virus vaccine</td>
<td>Lanzhou Institute of Biological Products (LIBP), China</td>
<td>monovalent lamb rotavirus strain (G10P[12])</td>
<td>China, private market</td>
</tr>
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</table>
India (Bhandari et al., 2014). RVV has a lower efficacy in sub Saharan Africa and Asia, but the rotavirus attack rate and mortality burden in developing country settings is also much higher. This means that despite having a reduced vaccine efficacy, significantly more child deaths are prevented upon RVV introduction in a developing country setting than in a developed country setting (Lopman et al., 2012). This rationale led the WHO to recommend that rotavirus vaccines be included in all national immunization programs globally in 2009 (World Health Organization (WHO)).

Introduction of rotavirus vaccines into countries’ national vaccination programs have dramatically reduced rotavirus gastroenteritis (Desai et al., 2012; Bar Zeev et al., 2015) as well as diarrheal emergency department visits, hospitalizations (Cortes et al., 2011; Desai et al., 2012; Platts-Mills et al., 2017; Shah et al., 2016) and childhood diarrheal deaths (Enane et al., 2016; Richardson et al., 2011) across numerous countries. The path from RVV development to global RVV roll-outs and confirmation of this magnitude of effectiveness is a public health triumph. However, RVV impact could be even higher. Post-licensure effectiveness data have confirmed the gap in vaccine performance between developing and developed country settings. A meta-analysis evaluated RVV post-licensure vaccine effectiveness data from 24 countries. When countries were segregated by under-5 mortality rates, mean RV1 effectiveness was 84%, 75%, and 57% for countries with low, middle, and high child mortality, respectively (Jonesteller et al., 2017). Therefore, while currently licensed rotavirus vaccines have significantly reduced childhood gastroenteritis morbidity and mortality, the vaccine is not adequately protecting those children most at risk of dying. Further improvements in rotavirus vaccine efficacy could likely prevent tens of thousands of additional deaths per year in developing countries with high under-five mortality rates (Patel et al., 2012).

**Known determinants of rotavirus vaccine underperformance**

Improving RVV impact requires urgent identification of modifiable etiologies of the poor RVV performance in developing country settings. Numerous factors are already known to correlate with diminished RVV performance and causality is very likely multi-factorial. Here follows a brief overview of the major factors hypothesized to alter RVV performance in developing country settings, followed by a rationale for the hypothesis underpinning this thesis – namely that the infant intestinal microbiome composition is a major determinant of the reduced RVV efficacy observed in developing countries.
High maternal antibody titers to rotavirus in developing countries may diminish rotavirus vaccine take by interfering with vaccine strain replication. Higher pre-vaccination titers of both breast milk anti-RV IgA (Chilenga et al., 2016; Moon et al., 2010) and trans-placentally acquired anti-RV IgG (Appaiahgari et al., 2014; Moon et al., 2016) correlate with diminished RVV immunogenicity in several countries. Three randomized intervention studies built on these findings and tested if transiently withholding breast milk directly prior to vaccination could improve RVV seroconversion (Ali et al., 2015; Groome et al., 2014; Rongsen-Chandola et al., 2014). None of the studies demonstrated an improvement in RVV response. (Table 3) Hypothesizing that the effect of maternal antibodies might wane at older ages, additional studies have compared delayed or additive RVV dosing schedules. Results depended on location – with no change in immunogenicity in Pakistan and a 14% gain in seroconversion in Ghana when three RV1 doses were used instead of two (Ali et al., 2014; Armah et al., 2016; Colgate et al., 2016) (Table 3).

Additional research supports a correlation between RVV and secretor and histo-blood group antigen (HBGA) expression. Rotavirus has two major surface proteins – a spiked protease-sensitive P protein (VP4) and a glycoprotein G (VP7). Some rotaviruses may recognize HBGAs in a P-genotype dependent manner. An association between salivary HBGA expression and RV1 (a G1P[8] rotavirus strain) vaccine seroconversion were tested in an infant cohort in Pakistan and showed that RVV seroconversion rate varied significantly by salivary HBGA phenotype. Non-secretors (not expressing carbohydrates synthesized by FUT2) had the lowest seroconversion, while secretors with O blood group had the highest seroconversion (Kazi et al., 2017). This suggests that vaccine P[8] rotavirus strains may, for example, interact with H type 1 and Lewis b oligosaccharides, although in vitro data supporting such a mechanism has not been consistent (Sun et al., 2016). HBGA expression is likely only one of several factors affecting RVV immunogenicity, as developed countries have close to 95% RVV efficacy, despite having heterogeneous HBGA phenotypes in the population. Additionally, HBGA phenotype is an intrinsic genetic characteristic, and therefore not easily amenable to intervention.

Numerous studies have evaluated if nutrient and micronutrient deficiencies, malnutrition or HIV correlate with reduced RVV immunogenicity in developing countries (Colgate et al., 2016; Gastañaduy et al., 2016; Levin et al., 2017; Perez-Schael et al., 2007). These studies having conflicting results, and show no strongly
significant correlations with RVV immunogenicity. Serum zinc has been the most promising, correlating significantly with both RVV seroconversion and efficacy in Bangladesh. However, an intervention study evaluating the effect of zinc supplementation alone prior to vaccination in Indian infants was unable to demonstrate an increase in RVV seroconversion (Lazarus et al., 2017). Probiotics prior to RVV administration in combination with zinc or alone show modest improvements in RVV seroconversion (Isolauri et al, 2003; Lazarus et al. 2017) (Table 3).

Finally, co-administration of both bi and tri-valent oral polio vaccine (OPV) and RVV has been shown to modestly reduce RVV seroconversion by 5-15% (Emperador et al., 2016; Ramani et al., 2016; Steele et al., 2010), (Table 3), possibly through viral competition (Wang et al., 2012). Ameliorating this interaction is difficult as OPV use will depend upon the timing of the Global Polio Eradication Campaign. Currently inactivated polio vaccine (IPV) is being introduced and trivalent OPV is being replaced by bivalent OPV.

While all these factors may explain a component of the diminished RVV efficacy in developing countries, they in aggregate fail to adequately account for how RVV efficacy and effectiveness can be as low as 30-40% in some settings (Ali et al., 2014). An underexplored hypothesis explaining rotavirus vaccine performance is that the intestinal microbiome composition may be modulating RVV immunogenicity. Because manipulation of the microbiome is becoming increasingly feasible, identifying robust correlations between the microbiome composition and RVV response opens avenues to improving RVV performance in those settings where children are disproportionately burdened with rotavirus disease. Testing this hypothesis is the main aim of this thesis.

The intestinal microbiome and rotavirus vaccine underperformance

The term microbiome was first used by the Nobel Laureate Joshua Lederberg, who described it as the ‘collective genome of our indigenous microbes’ (Hooper and Gordon, 2001). In the last 15 years, application of next-generation sequencing techniques derived from the environmental sciences has revolutionized the understanding of these indigenous microbes. The human organism contains approximately $10^{14}$ cells, of which only 10% are animal – the rest are bacteria, archaea, fungi, and viruses and largely reside in the human intestinal tract (Harris et al., 2017; Savage, 1977). These microbes have co-evolved with humans and their mammalian ancestors and there is rapidly expanding evidence of their roles in human health and disease. (See list of definitions, Table 2). Chapter Two of this
thesis provides an overview of the known roles that the intestinal microbiome plays in clinical infectious diseases.

A set of four key findings support a role for the intestinal microbiome in determining diminished RVV immunogenicity in developing countries:

1. Across all ages, the intestinal microbiome differs by geography.
2. The intestinal microbiome shapes and regulates infant immunity.
3. Enteric viruses are known to interact with the bacterial microbiome.
4. The intestinal microbiome can alter vaccine responses in mouse models.

(1) The intestinal microbiome differs by geography across all ages:

In 2012, a key article (Yatsunenko et al., 2012) described differences in bacterial microbiome composition between healthy children and adults in the United States, rural Malawi, and Amazonas in Venezuela. At all ages the microbiome composition and functional gene reservoirs differed significantly between the US and both developing country populations. The study also described the development of the infant microbiome over time: regardless of geography –infants begin with low intra- and high inter-individual variability and move progressively towards higher intra-individual diversity with lower inter-individual differences or an ‘adult’ state over time. All factors driving microbiome maturity and geographic differences are not known, but an infant’s microbiome is shaped early by genetics, a mother’s own microbiota, mode of delivery (vaginal or caesarian), antibiotic use, and feeding practices (breast-feeding, formula, type and introduction of solid foods) (De Filippo et al., 2010; Duranti et al., 2017; Milani et al., 2017). In poor countries, undernutrition and repeated exposure to fecally-contaminated foods in combination with a high burden of eukaryotic disease likely significantly impact the microbiome over time (Blanton et al., 2016; Subramanian et al., 2015). There

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Microbiome</td>
<td>All microorganisms, commensal, symbiotic or pathogenic that share our body space</td>
</tr>
<tr>
<td>Bacterial Microbiome</td>
<td>All bacteria that share our body space</td>
</tr>
<tr>
<td>Virome</td>
<td>All viruses that share our body space, both those that can infect bacterial cells and those that can infect eukaryotic cells</td>
</tr>
<tr>
<td>Mycobiome</td>
<td>All fungal communities that share our body space</td>
</tr>
</tbody>
</table>
(2) The intestinal microbiome shapes and regulates infant immunity
It has long been known that presence of an intestinal microbiome is essential for normal immune development. The clearest example of this is the aberrant immune development observed in germ-free mice, who do not have normal gut-associated lymphoid tissue (GALT) and IgA B cell maturation (Bouskra et al., 2008; Kunisawa et al., 2013). The resident intestinal bacteria interact with the innate and adaptive immune system and are important regulators of the immune steady state and readiness for infection (Collins and Belkaid, 2017; Honda and Littman, 2016; Kamada and Núñez, 2014). Therefore, geographic differences in key microbial taxa could potentially influence the maturation and regulation of infant immunity and responses to the live RV vaccine strain.

(3) Enteric viruses are known to interact with the bacterial microbiome
Enteric viruses have been shown to engage in a unique triangulation with the bacterial intestinal microbiome and the immune system once they enter the human intestinal tract (Pfeiffer and Virgin, 2016). Poliovirus, mouse mammary tumor virus, and norovirus as well as murine rotavirus have all been shown to interact with the intestinal microbiome (Baldridge et al., 2014; Kane et al., 2011; Kuss et al., 2011; Robinson et al., 2014; Uchiyama et al., 2014). Specifically, study of murine rotavirus showed that microbiome depletion (either in gnotobiotic mice or through antibiotics) significantly delayed RV infection and reduced RV infectivity (Uchiyama et al., 2014). Enteric viral – virome interactions have also been described for rotavirus. In Bangladesh, the presence of non-polio enteroviruses in stool prior to vaccination correlated with a diminished RVV seroconversion, suggesting competitive viral replication (Taniuchi et al., 2016; Wang et al., 2012). These findings are not surprising when considering the millions of years of co-evolution between enteric viruses, resident bacteria, and human immunity. There is therefore an experimental precedent for viral-bacterial-immune responses underpinning a RVV-microbiome-immune response interaction.

(4) The intestinal microbiome can alter vaccine responses in mouse models
Finally, one set of experimental evidence supports a role for the microbiome in systemic vaccination (Collins and Belkaid, 2017; Littman, 2017; Valdez et al., 2014). In a mouse model, normal antibody response to the seasonal influenza vaccine required the presence of resident bacteria or flagellated E. coli, which directly stimulated plasma cell differentiation (Oh et al., 2014). These results sug-
Table 3: Examples of interventions to improve RVV immunogenicity and their effect on anti-RV IgA seroconversion.

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Vaccine</th>
<th>Country</th>
<th>Intervention</th>
<th>anti-RV IgA seroconversion</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probiotic</td>
<td>DxRRV</td>
<td>Finland</td>
<td>Lactobacillus casei GG</td>
<td>74% w/o vs 93% with p=0.05</td>
<td>(Isolauri, 2003)</td>
</tr>
<tr>
<td>Probiotic and Zinc</td>
<td>RV1</td>
<td>India</td>
<td>Probiotic alone</td>
<td>NS</td>
<td>(Lazarus, 2018)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Zinc alone</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Probiotic and zinc</td>
<td>12.0% increase (CI,0.8, 22.8), p=0.04</td>
<td></td>
</tr>
<tr>
<td>Delayed or added dosing</td>
<td>RV1</td>
<td>Bangladesh</td>
<td>10 and 17 weeks, no control</td>
<td>73.5% (CI,45-87%) (no comparator)</td>
<td>(Colgate, 2016)</td>
</tr>
<tr>
<td></td>
<td>RV1</td>
<td>Ghana</td>
<td>6/10 vs 6/10/14</td>
<td>28.9% vs 37.4%, p=0.014</td>
<td>(Armah, 2016)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6/10 vs 10/14 wks</td>
<td>28.9% vs 43.4% p=0.163</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RV1</td>
<td>Pakistan</td>
<td>6/10 wks</td>
<td>36.1% (CI, 29.0, 43.9)</td>
<td>(Ali, 2014)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6/10/14 wks</td>
<td>36.7% (CI, 29.8, 44.2)</td>
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<td></td>
<td></td>
<td></td>
<td>10/14 wks</td>
<td>38.5% (CI, 31.2, 46.3)</td>
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<td></td>
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<td></td>
<td>All comparisons NS</td>
<td></td>
<td></td>
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<tr>
<td>Withhold breastfeeding (BF)</td>
<td>RV1</td>
<td>India,</td>
<td>Withhold 30 min pre/post RVV dose</td>
<td>NS</td>
<td>(Rongsen-Chandola, 2014)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6/10/14 wks</td>
<td>Increased in immediate BF arm:</td>
<td>Increased in immediate BF arm:</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>△-12.5% (CI,-21.2,-3.8) p=0.005</td>
<td>△-12.5% (CI,-21.2,-3.8) p=0.005</td>
<td>(Ali, 2015)</td>
</tr>
<tr>
<td></td>
<td>RV1</td>
<td>South Africa</td>
<td>Withhold 1 hour pre/post RVV dose</td>
<td>NS</td>
<td>(Groome, 2014)</td>
</tr>
<tr>
<td>Intervention</td>
<td>Vaccine</td>
<td>Country</td>
<td>Intervention</td>
<td>anti-RV IgA seroconversion</td>
<td>Ref</td>
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<tr>
<td>Staggered OPV - RRV</td>
<td>RV1</td>
<td>Bangladesh</td>
<td>RRV w/OPV</td>
<td>56.5% (CI, 44%, 68%)</td>
<td>(Zaman, 2009)</td>
</tr>
<tr>
<td>administration</td>
<td></td>
<td></td>
<td>RRV w/o OPV</td>
<td>66.7% (CI, 54%, 78%)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>RV1</td>
<td>Chile</td>
<td></td>
<td>RRV w/bOPV</td>
<td>50% (CI, 42%, 58%)</td>
<td>(Ramani, 2016)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>RRV w/IPV</td>
<td>65% (CI, 60%, 71%)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>p=0.004</td>
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<tr>
<td>RV1</td>
<td>Bangladesh</td>
<td></td>
<td>RRV w/OPV</td>
<td>47% (CI, 39%, 54%)</td>
<td>(Emperador, 2016)</td>
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<tr>
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<td></td>
<td>RRV ≥1 d staggered w/OPV</td>
<td>63% (CI, 57%, 70%)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>p=0.001</td>
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Abbreviations: BF, breastfeeding; RV1, Rotarix; DxRRV, rhesus human reassortant vaccine; OPV, oral polio vaccine; bOPV, bivalent OPV; IPV, inactivated polio vaccine; wks, weeks; NS, not significant; CI, 95% Confidence Interval; Ref, reference.
gest that resident microbial taxa can stimulate vaccine-induced immunity, acting similarly to an adjuvant for a systemic vaccine.

These four lines of evidence formed the rationale for embarking upon the work presented in this thesis.

**Aims and Outline of this thesis**

The central aim of this thesis is to test the hypothesis that the intestinal microbiome modulates RVV immunogenicity and thereby contributes to the diminished RVV efficacy observed in developing country settings.

**Part I – Background**

*Chapter Two* aims to give an overview of the known roles for the microbiome in clinical infectious diseases. This provides a background for understanding the microbiome’s relevance to early immune development as well as enteric infections.

**Part II – Correlations of microbiome composition and RVV immunogenicity**

If the microbiome modulates RVV immunogenicity, then there must be differences in microbiome composition between infants who do and who do not seroconvert to RVV in developing country settings.

*Chapter Three* tests whether there are bacterial microbiome differences prior to vaccination between RVV responders and non-responders in an urban slum in Karachi, Pakistan.

*Chapter Four* tests whether there are bacterial microbiome differences prior to vaccination between RVV responders and non-responders in rural Navrongo in northern Ghana.

*Chapter Five* provides a more extensive and temporal analysis of microbiome and RVV correlations, testing whether there are differences not only in bacterial microbiome, but also virome and mycobiome composition over time between RVV responders and non-responders in northern Ghana.

**Part III – From correlation to causation**

If the microbiome modulates RVV immunogenicity, then modulating the microbiome should alter RVV immunogenicity.
Chapter Six describes establishment of a novel mouse model to test the effect of modulation of the microbiome on RVV immunogenicity \textit{in vivo}.

Chapter Seven describes a human volunteer study whose aim was to evaluate the effect of antibiotic modulation of the intestinal microbiome on RVV immunogenicity.

\textit{Part IV – From causation to intervention}

Chapter Eight provides a synthesis of the data presented in this thesis with suggestions for future work to establish if a microbiome-based intervention can improve RVV performance and decrease rotavirus-related deaths in developing country settings.
REFERENCES


General Introduction


General Introduction


