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Socioeconomic and Environmental Exposures and Their Associations with Vaccine Response in Young Children in Northern Ecuador

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Bachelor of Arts
Concordia College
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Abstract

Socioeconomic and Environmental Exposures and Their Associations with Vaccine Response in Young Children in Northern Ecuador
By Alexandra Buck

This study measured the association between socioeconomic and environmental exposures and vaccine response to nine childhood vaccines. The association was measured using birth cohort data from the ECUAVIDA (Ecuador Life) study conducted in Quinindé, Esmeraldas Province, Ecuador. 2,404 neonates and their mothers were recruited between 2006 and 2009. Criteria for inclusion in the analysis required having received the full vaccine series with respect to each vaccine, with the exception of the rotavirus vaccine. Data analysis used SAS version 9.4. Logistic regression was used to model the association between the exposures and the odds of meeting the achievement of a designated protective antibody threshold (seropositivity) for each vaccine. Linear regression was used to model the association between the exposures and the log-transformed antibody titers, resulting in a geometric mean antibody titer (GMT) ratio measurement. Socioeconomic status (SES) was found to be a significant predictor for the rotavirus GMT model (-0.16, 95% CI: -0.31, -0.01). A slightly protective association was found between low SES and increased rotavirus GMT ratio. Because antibodies generated from rotavirus vaccine and natural exposure cannot be distinguished from one another, it is possible that children who are lower SES could have more natural exposures, resulting in higher antibody titers being detected. Future research should focus on the biological mechanisms that broader societal factors, such as low SES, act through to impact vaccine response.

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Chapter 1: Background/Literature Review

Vaccines are incredibly important public health interventions to prevent infectious diseases, especially in young children. They have saved countless lives and continue to improve in efficacy and coverage. However, in developing or low-income countries, efficacy for vaccines, especially oral vaccines, tends to be lower where they are usually needed the most (1). Oral vaccines such as rotavirus and poliovirus vaccines are crucial in protecting children from enteric infections and have high efficacies in high- and middle-income countries (1). Both rotavirus vaccines, Rotarix and RotaTeq have pooled efficacy estimates of 73% in industrialized countries but have significantly lower pooled estimates of 20% in developing countries (2). Similarly, rates of seroconversion after trivalent oral poliovirus vaccination are almost 100% in industrialized countries but are only 73% and 70% in developing countries for poliovirus type 1 and 3, respectively (3).

Because oral vaccines interact with the mucosa of the digestive tract, it is believed that factors that can interfere with mucosal surfaces may play a role in the differential effects seen in oral vaccine efficacy in developed versus developing countries. Researchers have offered many potential explanations such as undernutrition, microbial overload on mucosal surfaces of the digestive tract, alteration of the microbiome as well as maternal antibodies in serum and/or breast milk that may alter a child's mucosal pathology and lower immune response to interventions using oral vaccines (1). This literature review aims to discuss ways in which vaccines are different in developing versus developed countries as well as how the many different hypotheses used to explain these differences have been demonstrated in past research and may be interrelated with one another. Specifically, how factors such as malnutrition, animal exposure, bathroom

environment, concurrent infections and maternal antibodies impact vaccine response in young children in developing countries will be discussed.

Vaccines in Developing Countries

Reduced Vaccine Efficacy

Besides oral rotavirus and poliovirus vaccines, many other oral vaccines such as typhoid and cholera have been examined in developing countries where their integration is greatly needed to prevent a high burden of enteric disease but generally where lower efficacies are observed (4, 9). A prospective cohort study that examined differences in oral cholera vaccine response in Swedish versus Nicaraguan children demonstrated that while Swedish children generally had lower antibody levels prior to vaccination, which is likely due to limited exposure, Swedish children had higher serum responses to the oral cholera vaccine than Nicaraguan children did, reinforcing past studies that have shown lower oral vaccine efficacies in developing countries when compared to developed countries (1, 2, 3, 4). Researchers hypothesized that these results may be due to small bowel bacterial overgrowth that has been observed in past studies on children in developing countries (4). Bacterial overgrowth as well as environmental enteropathy is a large concern in regards to oral vaccine efficacy in developing nations.

Environmental enteropathy is a subclinical condition where constant fecal-oral contamination results in the blunting of intestinal villi and intestinal inflammation (5). Environmental enteropathy is believed to impact oral vaccine response because it causes chronic immune system stimulation which may reduce a child's ability to respond to the vaccine and because it damages the mucosal surface which many oral vaccines attach to as part of their biological mechanisms (5). Oral rotavirus vaccine, for example, is

generally used in areas that have a high burden of disease not only for rotavirus but also for other enteric diseases which subsequently can cause high viral challenge loads in children receiving the vaccine (6). Living in areas with a high microbial burden is believed to be associated with low socioeconomic status, risk of environmental enteropathy and lower oral vaccine immune response which may in part explain why countries like Malawi have a rotavirus vaccine efficacy of 49% and other high-income countries have efficacies of 95% (7). Environmental enteropathy can also impact the way researcher's measure vaccine efficacy if the gut where vaccines are interacting is damaged. For instance, the correlate of protection for rotavirus vaccine is serum IgA which scientists argue may be a poor measure of protection because it may not sufficiently capture the relevant gut immune responses or predict protective efficacy (6, 8). Therefore, the way vaccine responses are measured as well as environmental risk factors, including environmental enteropathy, may impact the poor oral vaccine efficacy seen in developing countries.

Vaccine Coverage and Barriers to Vaccine Uptake

Another concern regarding vaccines in developing countries is vaccine coverage, which remains an issue in developing countries due to cost, healthcare access and communication (9, 10). Studies have found various ranges of coverage which can differ by vaccine. In Kolkata, India, the coverage for the Vi typhoid vaccine was 61% while the Pediatric Vaccine-Preventable Diseases Working Group Meeting found that in all the regions represented at the meeting, vaccination for MMR, in conjunction with or independent of varicella, was not in use in the public sector (9, 10). This meeting also found issues with pertussis booster vaccination coverage and found the main barriers to

be vaccine registration and cost, which makes completing the childhood vaccination program in many low resource regions difficult (10).

Lower efficacy in high burden countries, which generally consist of developing countries, is another factor that may hinder national leaders from introducing certain vaccines into national immunization programs, subsequently reducing coverage (11). For example, by the end of 2011, none of the developing countries in the Southeast Asian and sub-Saharan Africa region, with the exception of Sudan, had implemented the rotavirus vaccine into their national immunization programs due to a perceived lack of need as well as evidence from vaccine trials that demonstrate low efficacy in high burden areas (11). Ministries of health as well as healthcare personnel have also described the challenges of delivering large capacities of the vaccine due to its cold chain requirements and its cost (11). Some nations have received GAVI support that has reduced the cost of one rotavirus vaccine dose to below one US dollar but for countries who do not have this financial support, it is unlikely they would implement the vaccine into the national immunization program at any cost above one US dollar, reducing coverage in countries that likely need an increase to reduce the overall burden of disease (11).

Other Challenges

Issues such as low socioeconomic status, environmental enteropathy, low access to healthcare and overall reduced efficacy in high disease burden countries are some of the barriers to successful vaccination in developing countries. However, other factors that can impact successful vaccination include malnutrition, high environmental microbial burden from animal exposure, latrine use, water sanitation and concurrent infections, breastfeeding and maternal antibodies (elaborated in the sections that follow). These and

other factors make measuring vaccine impacts challenging and should be accounted for when analyzing vaccine effectiveness in young children in developing countries. It is possible that some of these factors make a larger impact than others; malnutrition and environmental microbial burden likely play a significant role in successful oral vaccine protection due to the gut-immune response relationship that is involved with oral vaccines.

Malnutrition and Vaccine Response

Malnutrition is a large contributor to mortality around the globe and is also a concern for oral vaccine response because it impacts the gut microflora and subsequently, the gut immune system that oral vaccines act upon. A large cohort study examined infants from an urban slum in Dhaka, Bangladesh to identify risk factors for poor response to oral poliovirus vaccine (OPV) and found one of the greatest risk factors to be malnutrition as well as diarrhea and shorter breastfeeding duration (12). Researchers found that having a weight-by-age z-score less than negative two was significantly associated with having low OPV 3 titers (12). Authors concluded that their study results support the hypothesis that a defect in induction immunity in the gut for OPV is what leads to decreased oral vaccine response in developing countries (12). Another multi-site cohort study examined differences in rotavirus infections based on the presence or absence of rotavirus vaccination programs and found that a significant protective factor against rotavirus infection and disease was having higher weight at the first month of life (13). This study also identified major risk factors for infection as increasing maternal age, lower socioeconomic status and the presence of overcrowding in the family (13). While

these risk factors do not directly include malnutrition, it is indicated that nutrition, weight and income are all important factors in vaccine effectiveness.

Researchers have also aimed to identify the specific biological mechanisms that make malnutrition an important factor in immune development and responses. An experimental research study aimed to determine what factors contribute to mortality due to rotavirus diarrhea by using gnotobiotic pigs transplanted with the fecal microbiota of a healthy two month old human (14). The pigs were fed either a protein-sufficient or –deficient diet and then infected with virulent human rotavirus (14). Researchers found that protein-deficient pigs had decreased rotavirus antibody titers and total IgA concentrations, systemic T helper and cytotoxic T lymphocyte frequencies as well as serum tryptophan and angiotensin I-converting enzyme 2 which demonstrate a compromised adaptive immune response as well as amino acid homeostasis (14). The authors then hypothesized that in areas where malnutrition and rotavirus disease are prevalent, interventions that include protein supplementation and/or immunomodulatory probiotics may increase the immune response to rotavirus infection (14). Because researchers hypothesize that diet impacts the immune response to infection, it is highly likely that diet and malnutrition also impact the body's ability to respond to vaccines.

While many research studies have demonstrated how malnutrition may impact immune responses and subsequently responses to vaccines, other studies have examined how vaccines may aid in preventing malnutrition. Because vaccines reduce the amount of recurrent infections that children experience, the pathogenesis that leads to malnutrition may be diminished by being vaccinated (15). However, because many vaccinations are given in the early months of life and malnutrition has still been observed as a risk factor

for reduced vaccine response, it is likely that malnutrition has a negative impact on vaccine response early in life, not vice versa (12, 13).

Environmental Microbial Burden

Animal Exposure

Interacting with animals is a large part of life for many people around the world and has many benefits. However, animals also present the possibility of contracting zoonotic infections, developing allergies and introducing higher loads of microbes into the human environment in general. Because stimulation of the immune system and high microbial burden can negatively impact vaccine response, continually exposure to animals may in turn impact vaccine response in young children. Increases in a child's environmental microbial burden may be a result of interacting with animals and subsequently, contracting zoonotic infections from animals. One group of researchers aimed to determine how owning a pet dog or cat can increase an owner's chance of contracting *Campylobacter jejuni/coli* and using source-attribution and case-control analysis, researchers found that owning a dog, particularly a puppy, significantly increased the risk of infection with pet-associated sequence types (16). Researchers found significantly more exact matches of owner-pet sequence types than was expected and discussed how the high degree of overlap between human and pet *C. jejuni/coli* sequence types suggests some type of significant interaction between owners and pets (16). The zoonotic risk characterized by this research demonstrates how animal exposure, specifically pet exposure, can increase risk of infection.

Another way in which animals can impact human health is through exposure to allergens. Exposure to allergens can cause symptoms such as wheezing and asthma and

can also cause chronic stimulation of the immune system. If exposure to animal allergens chronically activates the immune system, a reduction in vaccine response may be possible. A population-based birth cohort study conducted in the United Kingdom aimed to investigate whether pet ownership during pregnancy and early childhood was associated with wheezing from birth to seven years old (17). Through logistic regression, researchers found that cat ownership was significantly associated with an overall 6% lower odds of wheezing from birth to seven years old while rabbit and rodent ownership was significantly associated with a 21% and 11% higher odds of wheezing, respectfully (17). Dog ownership and bird ownership had no association with wheezing episodes in this study (17). The presence and reactivity of animal allergens may impact vaccine response because the immune system is already stimulated at an abnormal level, indicating that animal exposure may be an important factor to consider when analyzing vaccine response.

Having high exposure to animals may also indicate being exposed to agricultural environments. Children who live in agricultural settings and those that live in urban settings have different levels of exposure to many things as well as different ecological factors that may impact their health. Those that live in agricultural areas may have higher exposure to organophosphate pesticides, endotoxin and allergens (18). Endotoxin and allergens stimulate maturation of the immune response early on in life and may impact long-term health as well as vaccine response (18). A longitudinal birth cohort study in Salinas Valley, California determined how different levels of these exposures in agricultural settings may impact Th1 and Th2 cytokine levels (18). Researchers found that mean Th2 levels were significantly higher in children with diagnosed asthma and

children with wheezing at two years of age (18). They also found that exclusive breastfeeding and pet ownership were associated with significant increases in Th1 levels while maternal agricultural work and the presence of a gas stove in the home were associated with significant increases in Th2 levels (18). Researchers concluded that the factors listed above all contributed significantly to the development of children's Th1 and Th2 immune responses (18).

Because animal exposure can impact a child's immune system in a variety of ways, it is plausible that it would also impact vaccine response. Whether or not a child is continually exposed to animals in their immediate environments at a young age could affect how strong or weak the immune system's response to a vaccine is. Because it is hypothesized that high microbial burden and immune stimulation can decrease vaccine response, particularly for oral vaccines, animal interaction could potentially decrease vaccine response in children who are routinely exposed to them at a very young age when their immune systems are still developing and the majority of vaccines are administered (4, 5, 6, 7).

Water and Sanitation

In many developing countries, adequate water and sanitation are among the top goals for improving population health. Having enough clean water and having access to proper sanitation is a large issue in many places and can impact many facets of health. For example, some enteric infections have preventative oral vaccines but these vaccines have been shown to have lower effectiveness in places where issues concerning sanitation, water and hygiene are present (19). Researchers believe that poor sanitation negatively impacts vaccine effectiveness because of the increased fecal-oral bacterial

exposure that occurs early in life in many developing nations (20). Fecal-oral bacterial exposure can dampen the immune response to oral vaccines because the mucosal immune system is chronically activated (20). Researchers also believe that poor sanitation plays a large role in the prevalence of environmental enteropathy in developing countries, even more so linking poor sanitation to decreased oral vaccine response (5, 7, 20).

Poor sanitation can be defined in many ways and is usually categorized based on the prominent bathroom type a family uses. This can include a field, family latrine, shared latrine, toilet service, etc. Shared latrines are of large public health concern due to the potentially large number of people that use them and subsequently the large microbial load that individuals are then exposed to. Being exposed to multiple microbes in a small area can increase the risk of infection as well as potentially decrease vaccine response if chronically exposed. In a large systematic review, researchers identified shared facilities as a significant risk factor for adverse health outcomes when compared to individual household latrines (21). Another large case-control study also identified sharing sanitation facilities with multiple families to be a significant factor for moderate-to-severe diarrhea in the nations of Kenya, Mali, Mozambique, Pakistan and India (22). Another case-control study was conducted to examine the paralytic poliomyelitis outbreak in Taiwan in 1982 (23). The main risk factor identified in cases was being unvaccinated but researchers also found that if a family shared a latrine with other families, the odds of contracting polio was four times higher (23). Because poor sanitation increases the risk of infection, it likely plays a large role in vaccine response due to the large microbial load people are continually exposed to as well as the social and economic indications that are correlated with poor sanitation (24).

Other studies have identified even single family latrines to be a risk factor for disease (25). Two prospective cohort studies that examined the association between latrine use and risk of infection in Bangladesh and Ecuador found that the presence of a family latrine was associated with a higher risk of infection (25, 26). Another case-control study analyzing the different risk factors for contracting Cholera among vaccinated individuals in Haiti identified household water disinfectant products and latrines as the main household toilet as significant risk factors for cholera infection (27). Researchers found that while a household latrine was considered a private facility, the median number of people using one household latrine was 15, significantly increasing the risk of infection (27). The high microbial load that familial latrines in low income areas exposes individuals to increases their risk of infection significantly, potentially reducing their response to vaccines if they are constantly exposed to infectious agents.

Understanding how sanitation impacts health is complex because the association involves many behavioral, ecological and immunological factors. However, because poor sanitation is so widespread throughout developing nations where oral vaccines appear to be less effective, sanitation should be included when understanding poor vaccine performance. Poor sanitation is not only indicative of the type of social and economic factors a child is exposed to but also has a large influence on the amount of microbial pathogens a child may be exposed to starting at an early age, potentially negatively impacting vaccine response through environmental as well as immunological factors.

Concurrent Infections

Having multiple infections at one time is an issue that faces many children in the developing world. Because of their high exposure to pathogens and the social and

ecologic factors that they grow up in, having concurrent infections as well as chronic infections can impact vaccine response. A prospective cohort study examined the influence of co-infections on maternal and infant measles-specific IgG levels and found that infant malaria parasitaemia, infant HIV and infant wasting to be risk factors for decreased measles-specific IgG levels (28). This study is unique because in other studies that have examined the association between infections and vaccine response, the association was analyzed in school-age children not infants. For example, one review article examined many different studies that analyzed how infections can impact a child's vaccine response and found that intestinal helminthes may reduce responses to BCG vaccination in school-age children (29). They also found that for filarial infections, the response to tetanus toxoid was reduced (29). In another study, rural and urban children were compared to analyze influenza-specific antibody titers following vaccination and found lower responses in helminth-positive compared to helminth-negative children (29).

Because many of the associations seen in this review article were based off of helminth infections, the authors suggested that helminth and other infections may have a strong impact on vaccines that require a Th1 response in order to generate protective immunity (29). When an individual becomes infected with a helminth or other intestinal parasite, the immune system switches from a Th1 response to a Th2 to fight the infection, causing a reduction in the Th1 response (30). Th1 is used to fight off several viral and bacterial infections which may explain why geohelminth infections have been shown to have negative effects on vaccine immunity to oral as well as parenteral vaccines (30). Because of these findings, many scientists have suggested de-worming particularly

burdened populations prior to vaccination in order to increase vaccine response as much as possible (29).

A common antihelmitic drug that is used for de-worming is albendazole (31, 32). A randomized, controlled trail aimed to determine if albendazole increased vibriocidal antibody reponses to oral cholera vaccine in children infected with *Ascaris lumbricoides* (31). Researchers found that post-vaccination rates of vibriocidal antibody seroconversion were greater in the treatment group than in the placebo group but that this result was not statistically significant (31). After stratifying by blood type, researchers did find that seroconversion rates were significantly higher in the treatment group than in the placebo group but only for non-O blood type groups while seroconversion was greater in the placebo group for O blood types (31). Researchers explained that the O blood type group is associated with a more severe host response to cholera infection and has also been shown to have greater immunoresponsiveness to oral cholera vaccine compared to non-O blood type groups, which may explain the inverse statistical relationship observed (31).

Having an infection during the period of most frequent vaccination and/or having multiple infections at once have been shown to greatly impact vaccine response. Because many vaccines, especially oral vaccines, require a robust Th1 response in order to reach protective levels, infections with helminths and other parasites that cause the immune system to decrease the Th1 response in order to increase the Th2 response can greatly decrease vaccine efficacy (30). Also acknowledging how treatment of these infections can impact vaccine response is also important to consider as many studies have shown the benefits of treating children on vaccine response, even though this strategy may not

work as effectively in some groups of children compared to others (31, 32). The presence of concurrent and/or multiple infections as well as potential treatments should be considered when analyzing vaccine response, especially when examining children live in high burden areas of the world.

Interactions with Maternal Antibodies

Maternal Infections during Pregnancy

Because many childhood vaccines are given at a very early age, several during the first year of life, maternal antibodies that are passed through the placenta in-utero can impact vaccine response in infants. What the mother's diet is, the environment she is exposed to and her health can all affect the antibodies that are passed to the fetus during pregnancy. Chronic infections during pregnancy are one area of interest for studying how maternal antibodies influence a newborn's immune system development as well as many health outcomes such as susceptibility to infection and vaccine response. Research has shown that fetal adaptive immune responses are common in neonates who have been exposed to maternal infection during pregnancy (33).

A large prospective cohort study examined this association by studying mothers who had some type of infection during pregnancy and maternal as well as infant measles-specific IgG levels (28). Researchers found that 96% of mothers had protective measles-specific IgG levels and at delivery, the IgG levels in the infants' cord blood were positively correlated with maternal levels (28). However, when the infants were vaccinated for measles nine months later, researchers found that only 75% of infants had protective IgG levels which were much lower than the levels seen at delivery in the cord blood (28). Researchers believe that these differences are largely due to exposures the

infant experienced during the first nine months of life (28). This study demonstrates how maternal antibodies may have different impacts at different time points in an infant's life.

Another way in which maternal antibodies can impact differently is through different types of infections. An infection of major interest is maternal helminth infections, which have previously been shown to be associated with diminished vaccine responses in children (33). In a study performed in Kenya, it was observed that infants from mothers living in helminth endemic areas were able to generate cytokines to mycobacterial antigens prior to their vaccination for BCG (29). Another studying in Uganda demonstrated how maternal helminth infection is associated with a higher cytokine response to mycobacterial culture filtrate protein in one-year old infants (29). Research in Ecuador has also shown that plasma IgA levels specific to antigens in rotavirus and oral poliovirus vaccine were significantly higher in children of helminth-infected mothers compared to mothers who were not (34). Other intramuscular vaccines were also studied but vaccine antigens were comparable between the two groups, causing researchers to conclude that maternal helminth infections are not associated with reduced antibody responses to intramuscular vaccines but are associated with an increased IgA response to oral vaccines (34).

Because maternal helminth infections are of such interest on infant vaccine response, many studies have also been performed to analyze how treatment of these infections impact infant vaccine response. Clinical trial studies have found that cytokine responses were higher among infants born to mothers who were treated with antihelmitics compared to treatment with a placebo, demonstrating that helminth treatment may be beneficial for infant immune responses to subsequent helminth exposure as well as

vaccine response (29). However, other studies have found that antihelmitic treatment had no affect on infant responses to BCG, tetanus or measles vaccines (32). In this same study, it was found that if mothers were treated specifically for hookworm, then infants had reduced IL-5 and IL-13 response to tetanus toxoid (32). Due to differing results for how maternal antihelmitic treatments impact infants' vaccine responses, many researchers conclude that further studies are necessary to truly understand this association and the impacts on childhood health.

Breastfeeding

Another way that maternal antibodies can significantly impact an infant's immune system development and health in early life is breastfeeding. Breast milk allows for protection while infants' immune systems continue developing by providing maternal antibodies that the immature immune system cannot yet create in order to protect the infant from infection and other microbial exposures early in life. Many studies support the idea that breastfeeding has beneficial immunological effects on infants (35). A large multicenter study performed in Ghana, India and Peru found that exclusively or predominantly breastfed infants had a significantly lower risk of death from diarrheal and acute respiratory illnesses compared to those who were not predominantly breastfed (35). Another study in Dhaka, Bangladesh found similar results in that partial or no breastfeeding was associated with a significantly higher risk of death from acute respiratory tract infections as well as diarrheal illness when compared to exclusively breastfed infants (35). There is strong evidence that breastfeeding has highly beneficial effects on infant health and is therefore strongly recommended as an early life health intervention to decrease infant morbidity and mortality.

Whether or not breastfeeding is beneficial for infant vaccine response is another area of research, especially in regards to oral vaccines. Oral vaccines induce antigen-specific Th1/Th2 and IgA B cell responses simultaneously in the mucosal effector compartment because IgA is the major humoral effector in the mucosa (36, 37). Therefore, it is important to consider how breast milk impacts Th1/Th2 pathways as well as the IgA induction pathways to understand how it impacts oral vaccine response (36). One of the major factors that affect IgA production is transforming growth factor-beta (TGF-β) (37). TGF-β is a polypeptide that is responsible for epithelial cell growth and differentiation, development, carcinogenesis and immune regulation and is very important for the initial immunological development of infants (38).

In a study that examined TGF-β1 and TGF-β2 levels in maternal colostrum samples as well as IgA and IgM serum samples in newborns, researchers found substantial quantities of TGF-β1 and TGF-β2 in all colostrum samples (37). They also found a significant increase in serum IgA during the first month of life which was also significantly higher than the increase seen in IgM (37). The increase in serum IgA was highly and significantly correlated with levels of both TGF-β1 and TGF-β2 while IgM was only marginally correlated (37). Another study has shown how maternal TGF-β1 and TGF-β2 may play a role in maintaining homeostasis in the intestine of infants which helps to regulate inflammation in the digestive tract (39). In a second experimental study examining maternal cytokines in colostrum and their effect on infant's immune systems, researchers found that TGF-β1 and IL-4, which is another important cytokine, promoted the secretion of IgA but suppressed the B cell responses to two antigens (40). Researchers

hypothesized that these responses were to allow the commensal intestinal microflora to develop and to maintain homeostasis of the gut (40).

TGF-β is an important factor in infant's responses to oral vaccines because it promotes IgA secretion, which many oral vaccines induce for effective vaccine response (36, 37). Because various researchers have demonstrated that it is found in high concentrations in breast milk, there is a possibility that breast milk is associated with a proper immune response to oral vaccines (37, 39, 40). However, B cell responses are also important for effective vaccine response because of the memory that they induce for future exposure to infections and as some research has demonstrated, breast milk, particularly colostrum, may also have a suppressive effect on B cell responses because of TGF-β (40). There is not a definite answer on how TGF-β in breast milk impacts vaccine response.

TGF-β is not the only important immune factor that effects how breast milk is associated with vaccine response. Soluble CD14 is an anchored membrane protein on mature monocytes that functions as a coreceptor for bacterial lipopolysaccharide (LPS) and triggers the induction of inflammatory responses by the immune system in the presence of LPS (41). Researchers studied the impact of soluble CD14 on B cell growth and differentiation and found that it bypasses the physiological path that may limit B cell activation which effectively increases B cell activation (41). Because of this, researchers concluded that soluble CD14 may play a beneficial role in infant immunity by triggering B cell activation before fully functional T helper cells are developed to stimulate B cell activation (41).

Another study that examined the role of soluble CD14 found in human breast milk in innate immune responses in infants found that CD14 interacts with the intestinal epithelial cells of infants, producing many cytokines, growth factors and other immune factors when exposed to bacterial LPS (42). Researchers also studied the concentration of soluble CD14 in breast milk over time and found that the highest concentrations were during the first week postpartum followed by a steady decrease over time (42). Similarly to TGF-β, soluble CD14 appears to potentially be beneficial in enhancing vaccine response in infants but because it decreases almost directly after birth, these effects may not be present when infants begin receiving many of their vaccinations.

The association between breast milk and vaccine response is not fully understood and many research studies have resulted in contradictory observations. However, it is still a crucial aspect of immunity in infants and therefore should be considered when analyzing vaccine response in young children. Particularly in developing countries, breast feeding is one of the main early life interventions that can benefit an infant's immune development because it can aid in nutrition, passive immunity, exposure to beneficial microbes and many other positive health factors (35). Because of these positive impacts that breast milk has on early life health, it may be that while breast milk does not directly aid in vaccine response, it can help to create a healthier life overall for an infant, allowing for a more robust vaccine response. Breastfeeding is certainly necessary to consider when analyzing vaccine response.

Conclusion

As was demonstrated throughout this literature review, factors that can potentially cause a change in vaccine response is a complex and heavily researched issue,

particularly for oral vaccines in developing nations. Proximal factors that impact vaccine response, such as maternal antibodies, having concurrent infections, and malnutrition are important to study with a focus on the individual as biological processes are a major player in these factors. More distal factors that may impact vaccine response such as socioeconomic status, environmental microbial exposure from latrine use or animal interaction, and water contamination are also important to consider in broader epidemiological studies in order to understand why certain regions and populations of the world have decreased vaccine response than others. The subsequent epidemiological study aims to better understand some the associations between these distal socioeconomic and environmental factors and vaccine antibody responses in young children living in a developing country.

Chapter 2: Manuscript

Introduction

In developing or low-income countries, efficacy for vaccines, especially oral vaccines, tends to be lower than in middle- or high-income countries (1). For example, both rotavirus vaccines, Rotarix and RotaTeq, have pooled efficacy estimates of 73% in industrialized countries but have significantly lower pooled estimates of 20% in developing countries (2). Similarly, rates of seroconversion after trivalent oral poliovirus vaccination are almost 100% in industrialized countries but are only 73% and 70% in developing countries for poliovirus type 1 and 3, respectively (3). Because oral vaccines interact with the mucosa of the digestive tract, it is believed that factors that can interact with mucosal surfaces may play a role in the differential effects seen in oral vaccine efficacy in developed versus developing countries (5).

Researchers have offered many potential explanations such as malnutrition, microbial overload on mucosal surfaces of the digestive tract, alteration of the microbiome, and maternal antibodies in serum and breast milk that may alter a child's mucosal pathology and lower immune response to interventions using oral vaccines (1). A body of research has focused on factors that directly impact vaccine response such as maternal antibodies in a child's blood or gut from breast milk antibodies, a child's nutritional status and breast feeding duration (33, 12, 35). These phenomenons are generally not observed in intramuscular vaccines which generally work similarly in developed versus developing countries (9, 34).

However, there are also explanations beyond factors that directly affect the mucosa that may also alter oral vaccine response. Focusing on the socioeconomic and

environmental factors that may indirectly impact vaccine response is also important because they may be used to explain why population differences in vaccine response are observed. Living in areas with a high bacterial and parasitic microbial burden, which is believed to potentially decrease oral vaccine response, has been shown to be associated with low socioeconomic status (5). Other important factors such as malnutrition, unsanitary latrine use, and concurrent parasitic infections are also associated with SES and vaccine response (7, 13, 24, 28). Because low SES is associated with these factors that are in turn associated with more direct factors such as microbial overload on mucosal surfaces and the presence of maternal antibodies, it can by hypothesized that broader socioeconomic and environmental factors are the drivers behind what direct factors impact vaccine response.

This study aims to analyze how broader socioeconomic and environmental factors such as household crowding, maternal education, animal exposure and socioeconomic status are associated with several vaccine antibody response measurements in 13-month-old children in northern Ecuador, with particular emphasis on oral versus intramuscular vaccines.

Methods

Study Design and Population

Using data obtained through the ECUAVIDA (Ecuador Life) birth cohort study conducted in Quinindé, Esmeraldas Province, Ecuador, associations between various socioeconomic and environmental exposures and childhood vaccine antibody titer responses were analyzed. The IRB at Emory University provided a letter of exemption for this study. Quinindé is considered a rural area that is part of the tropical region on the

coast of Ecuador (30). 2404 neonates and their mothers were recruited between 2006 and 2009 into the cohort (30). The original study objective was to analyze how soil-transmitted helminthes and other early microbial exposures impact the development of atopy, allergic diseases and immune responses in young children (30). Data on socioeconomic and environmental factors were collected from the mother within two weeks of delivery and additional data on the child's health was collected at the 7 month follow-up visit (30). Vaccine response measurements were collected when the child was approximately 13 months old (30). Further information regarding the design and methods of the original cohort study can be found in Clark et al. and Cooper et al. (34, 30). Vaccine response was measured in two ways: achievement of a designated protective antibody threshold (seropositivity) and antibody titers (34). The antibody titers were log-transformed in order to measure a summary geometric mean antibody titer (GMT) for each exposure. The protective antibody thresholds for each vaccine are as follows: rotavirus IgA $\geq$ 20 U/mL; OPV3 IgA $\geq$ 3,100 mIU/mL; diphtheria toxoid IgG 0.01-0.1 IU/mL; tetanus toxoid IgG > 0.1 IU/mL; pertussis IgG > 5 units; HIB IgG > 0.15 mg/mL; measles IgG > 120 mIU/mL; rubella IgG 10-15 mIU/mL (30).

Analysis was conducted separately for each vaccine: rotavirus (Rotarix), diphtheria, tetanus, pertussis and Haemophilus influenza type B (HIB) in a pentavalent vaccine (Novartis), measles and rubella (MMR), and OPV1 and OPV3 in a trivalent OPV vaccine (Chiron). Criteria for inclusion in the analysis required having received the full vaccine series with respect to each vaccine, with the exception of the rotavirus vaccine (Table 1).

Table 1: Doses of Childhood Vaccines and their Associated Counts

	Dose	*n (%)*
Rotavirus		
	2*	830 (63%)
	1*	218 (16%)
	0	278 (21%)
Trivalent OPV		
	3*	1,569 (98%)
	2	22 (1%)
	1	1 (0.001%)
	0	3 (0.002%)
Pentavalent		
	3*	1,593 (100%)
	0	0 (0%)
MMR		
	1*	936 (61%)
	0	592 (39%)

*Required number of dose(s) for study inclusion

Table 1: Number of doses for each vaccine used in the study and what number of doses were required for study inclusion.

Rotavirus vaccine coverage was different from other vaccines in this analysis in that a larger proportion had not completed the full vaccine course (Table 1). This could potentially be due to the late introduction of rotavirus vaccine into Ecuador's immunization schedule relative to other vaccines, meaning that coverage is still increasing. Furthermore, it was found that approximately 50% of vaccinated subjects had met the designated rotavirus vaccine threshold measurement for either one or two doses. This is likely because rotavirus vaccine was recently introduced prior to the collection of vaccine response data. Because of this information, subjects who had received one or two doses of rotavirus vaccine were included in the analysis. MMR coverage was also modest at 13 months because per the vaccination schedule, children are not vaccinated until 12

months, indicating that some children may be slightly behind the vaccine schedule at the time of vaccine response measurement (30).

Statistical Analysis

To begin, all exposures available in the secondary data analysis were grouped into broad categories that are hypothesized to potentially impact childhood vaccine response, either directly or indirectly. The categories include socioeconomic factors, household characteristics, maternal factors, and child health. The theoretical relationships between categories and vaccine response are illustrated in Figure 1. Initial analysis to describe the frequency of all exposures for each vaccine and to identify patterns in significant exposures across vaccines for the seropositivity thresholds used a central Fisher exact test (Tables 2 and 3). The frequency of all exposures and summary GMT measures were also summarized (Tables 4 and 5). Significant exposures, defined by an alpha level less than 0.05, would then be included in statistical modeling, the objective of which was to identify significant predictors for meeting the seropositivity threshold and increasing the GMT.

No significant patterns in exposures across seropositivity thresholds were identified, leading to model construction that was based off of *a priori* identification of important exposures for vaccine response in an extensive literature review. Using Figure 1, variables that have been shown by previous research to be important factors in vaccine response, particularly for oral vaccine response, were chosen to represent each category (7, 18, 20, 28, 29). Socioeconomic status, household crowding, pet/farming animal exposure, sources of drinking water, maternal education, maternal vaccination during

pregnancy, child's sex and child's antibiotic use since birth (as a proxy for concurrent/recent infection) were chosen for the initial models.

Logistic regression was used to model the association between the exposures and the odds of meeting the seropositivity threshold for each vaccine. Linear regression was used to model the association between the exposures and the log-transformed antibody titers, resulting in a GMT ratio measurement. Stepwise backwards elimination with a p-value criterion of 0.20 or less for a predictor to remain in the model was then used to develop specific models for each vaccine as well as for both vaccine threshold and titer outcomes. A separate model was developed for each vaccine and outcome, resulting in fourteen unique models that were used for the final results of the analysis. All analyses were performed in SAS version 9.4.

Figure 1: Categories of Exposures and the Relationships to Other Categories and Vaccine Response

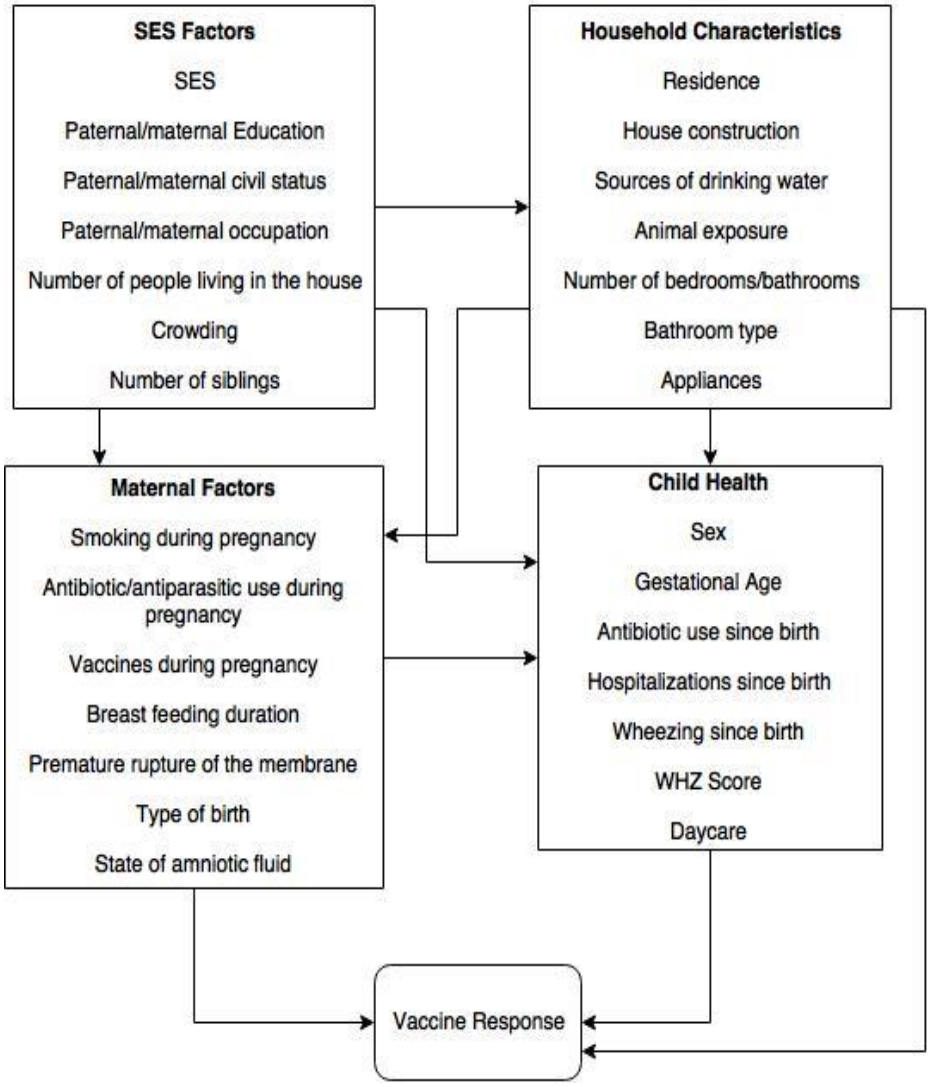

Figure 1: All available exposures grouped into broader categories to establish possible pathways in which childhood vaccine response may be impacted. Arrows indicate directionality.

Results

Few models kept the same predictors for each vaccine's response measurement type following stepwise backwards elimination. Most vaccine models either had slight or substantial variations in predictors used in both model types. The most consistent predictor in the seropositivity models was maternal vaccination during pregnancy, which appeared in three out of the eight vaccine models: OPV3, tetanus, and measles. The most consistent predictor in the GMT models was the child's use of antibiotics since birth, which appeared in four of the nine vaccine models: rotavirus, OPV1, tetanus, and pertussis.

SES, household crowding, the family's bathroom, the number of bedrooms in the house, and electrical appliances in the home were associated with rotavirus seropositivity (Table 2). No individual level or paternal variables were significantly associated. SES, household crowding and maternal education were included in the rotavirus seroposivity model. However, none of the predictors were found to be significant (Table 6). SES and child's antibiotic use since birth were included in the rotavirus GMT model and SES was found to be a significant GMT ratio (-0.16, 95% CI: -0.31, -0.01) (Table 7). For OPV3, maternal race/ethnicity and weight-by-height z-score were significantly associated with OPV3 seropositivity (Table 2). However, maternal vaccination during pregnancy was the only predictor included in the seropositivity model and it was not found to be significant (Table 6). Household crowding, SES, and maternal vaccination during pregnancy were included in the GMT OPV3 model but none were found to be significant predictors (Table 7).

The majority of significant variables for diphtheria seropositivity threshold were related to maternal characteristics (Table 3). However, the diphtheria seropositivity

model following stepwise backwards elimination resulted in no covariates remaining. In the diphtheria GMT model, pet/farming animal exposure and sources of drinking water were included but were not found to be significant (Table 7). For the tetanus seroposivity threshold, only the father's civil status was found to be a significant variable (Table 3). However, this was not included in the seropositivity model which contained SES, maternal vaccination during pregnancy and child's antibiotic use since birth, all of which were insignificant (Table 6). Child's antibiotic use and sources of drinking water were included in the GMT model but again were not found to be significant (Table 7).

The pertussis seropositivity threshold had wheezing since birth and attending day care as significant variables (Table 3). The seroposivity and GMT model included child's antibiotic use since birth, child's sex and pet/farming animal exposure but none were significant predictors in either model (Table 6, 7). The HIB seroposivity threshold had no significant variables in both univariate and multivariate settings (Table 3, 6). However, maternal education level was also found to be a significant GMT ratio for the HIB vaccine model when the level of education was either illiterate (-0.89, 95% CI: -1.68, -0.10) or some amount of secondary education (-0.34, 95% CI: -0.65, -0.03) (Table 7).

The measles seroposivity threshold found smoking during pregnancy and the type of birth to be significant predictors (Table 3). However, the seroposivity model included maternal vaccination during pregnancy and sources of drinking water, neither of which were significant (Table 6). The GMT model also included maternal vaccination during pregnancy but was not significant (Table 7). Significant variables for the rubella seroposivity threshold were paternal characteristics (Table 3). However, the results of

stepwise backwards elimination for rubella resulted in no remaining covariates for both the seroposivity model and the GMT model.

Table 2: Characteristics of the Achievement of a Designated Protective Antibody Threshold Following Infant Vaccination for Oral Vaccines

	Rotavirus, n/N (%) (n=807)	p-value	OPV3, n/N (%) (n=560)	p-value
Household & Environment				
SES				
Low	259/438 (59%)	**0.03**	179/375 (48%)	0.17
Medium to High	548/1,032 (53%)		381/875 (44%)	
Crowding				
Yes	339/579 (59%)	**0.02**	217/496 (44%)	0.56
No	468/891 (53%)		343/754 (45%)	
Family Bathroom				
Latrine	559/985 (57%)	**0.01**	394/840 (47%)	0.1
Toilet service	232/463 (50%)		160/395 (41%)	
Field	16/22 (73%)		6/15 (40%)	
Number of Bedrooms in the House				
1 to 2	591/1,036 (57%)	**0.01**	391/872 (45%)	1
3 to 7	216/434 (50%)		169/378 (45%)	
Electrical Appliances in the Home				
Refrigerator	522/986 (53%)	**0.03**	373/839 (44%)	0.76
Television	699/1,300 (54%)	**0.02**	495/1,108 (45%)	0.86
Stereo	363/677 (54%)	0.37	261/578 (45%)	0.82
Radio	310/578 (54%)	0.45	210/486 (43%)	0.38
Sources of Drinking Water				
Drinking	292/507 (58%)	0.17	200/431 (46%)	0.58
Piped	97/175 (55%)		74/148 (50%)	
Well	383/723 (53%)		262/612 (43%)	
River	16/31 (52%)		12/28 (43%)	
Rain	3/5 (60%)		1/5 (20%)	
Estuary	12/21 (57%)		8/20 (40%)	
Other	4/8 (50%)		3/6 (50%)	
Pet/farming animal exposure				
Yes	736/1,344 (55%)	0.78	515/1,152 (45%)	0.83
No	71/126 (56%)		45/98 (46%)	

Table 2 cont

	Rotavirus, n/N (%) (n=807)	p-value	OPV3, n/N (%) (n=560)	p-value
Mother				
Race				
Afro-Ecuadorian	212/356 (60%)	0.11	162/308 (53%)	**0.003**
Mixed	592/1,109 (53%)		397/939 (42%)	
Indigenous	3/5 (60%)		1/3 (33%)	
Civil Status				
Single	92/162 (57%)	0.66	65/137 (47%)	0.91
Married	118/222 (53%)		86/191 (45%)	
Divorced	4/8 (50%)		3/5 (60%)	
Widow	5/6 (83%)		2/5 (40%)	
Other	588/1,072 (55%)		404/912 (44%)	
Education				
Illiterate	13/21 (62%)	0.35	13/19 (68%)	0.23
Elementary	309/551 (56%)		212/473 (45%)	
Secondary	408/770 (53%)		288/650 (44%)	
Superior	77/128 (60%)		47/108 (44%)	
Smoking during pregnancy				
Yes	11/21 (52%)	0.83	8/17 (47%)	1
No	796/1,449 (55%)		552/1,233 (45%)	
Vaccines administered during pregnancy				
Yes	769/1,404 (55%)	0.48	533/1,196 (45%)	0.48
No	38/65 (58%)		27/53 (51%)	
Father				
Civil Status				
Single	53/95 (56%)	0.23	33/75 (44%)	0.97
Married	122/230 (53%)		90/195 (46%)	
Divorced	8/9 (89%)		4/8 (50%)	
Widow	1/1 (100%)		0/1 (0%)	
Other	593/1,078 (55%)		408/921 (44%)	
Education				
Illiterate	24/36 (67%)	0.11	20/33 (61%)	0.2
Primary	299/525 (57%)		202/452 (45%)	
Secondary	352/662 (53%)		248/561 (44%)	
Superior	59/123 (48%)		39/100 (39%)	
Child				
Sex				
Male	415/742 (56%)	0.43	287/636 (45%)	0.82
Female			273/614 (44%)	
Type of birth				
Vaginal	608/1,099 (55%)	0.8	428/931 (46%)	0.17
Cesarean	198/369 (54%)		132/317 (42%)	
Dystrophic	1/2 (50%)		0/2 (0%)	
Experienced wheezing since birth				
Yes	51/79 (65%)	0.1	32/67 (48%)	0.7
No	552/1,011 (55%)		393/866 (45%)	
Antibiotic use since birth				
Yes	236/408 (58%)	0.16	159/354 (45%)	1
No	571/1,062 (54%)		401/896 (45%)	
Day care				
Yes	23/33 (70%)	0.11	14/24 (58%)	0.22
No	584/1,063 (55%)		414/914 (45%)	
Weight-by-Height z-score				
Severe to Moderate Malnutrition	15/21 (71%)	0.17	13/20 (65%)	**0.04**
Average	377/700 (54%)		269/599 (45%)	
Overweight	12/27 (44%)		6/23 (26%)	

Table 3: Characteristics of the Achievement of a Designated Protective Antibody Threshold Following Infant Vaccination for Intramuscular Vaccines

	Diphtheria, n/N(%) (n=1,424)	p-value	Tetanus, n/N (%) (n=1,476)	p-value	Pertussis, n/N (%) (n=1,054)	p-value	HIB, n/N (%) (n=1,397)	p-value	Measles, n/N (%) (n=681)	p-value	Rubella, n/N (%) (n=824)	p-value
Household & Environment												
SES												
Low	412/420 (98%)	0.7	447/462 (97%)	0.11	313/454 (69%)	0.95	404/421 (96%)	0.68	171/219 (78%)	0.27	219/221 (99%)	0.26
Medium to High	1,012/1,036 (98%)		1,029/1,085 (95%)		741/1,078 (69%)		993/1,041 (95%)		510/624 (82%)		605/620 (98%)	
Crowding												
Yes	564/575 (98%)	0.59	592/621 (95%)	0.9	423/614 (69%)	0.96	557/579 (96%)	0.37	246/301 (82%)	0.65	303/306 (99%)	0.13
No	860/881 (98%)		884/926 (95%)		631/918 (69%)		840/883 (95%)		435/542 (80%)		521/535 (97%)	
Family Bathroom												
Latrine	938/965 (97%)	**0.02**	990/1,038 (95%)	0.81	714/1,027 (70%)	0.59	925/971 (95%)	0.62	437/545 (80%)	0.87	532/543 (98%)	1
Toilet service	466/470 (99%)		463/486 (95%)		325/481 (68%)		452/470 (96%)		235/287 (82%)		281/287 (98%)	
Field	20/21 (95%)		23/23 (100%)		15/24 (62%)		20/21 (95%)		9/11 (82%)		11/11 (100%)	
Number of Bedrooms in the House												
1 to 2	1,004/1,028 (98%)	0.7	1,049/1,100 (95%)	1	740/1,088 (68%)	0.33	992/1,037 (96%)	0.78	469/575 (82%)	0.4	566/575 (98%)	0.19
3 to 7	420/428 (98%)		427/447 (96%)		314/444 (71%)		405/425 (95%)		212/268 (79%)		258/266 (97%)	
Electrical Appliances in the Home												
Refrigerator	960/980 (98%)	0.57	990/1,035 (96%)	0.52	713/1,026 (69%)	0.41	949/995 (95%)	0.68	483/591 (82%)	0.29	573/587 (98%)	0.3
Television	1,273/1,303 (98%)	0.57	1,307/1,373 (95%)	0.34	943/1,361 (69%)	0.26	1,249/1,307 (96%)	1	618/761 (81%)	0.38	740/757 (98%)	0.4
Stereo	663/678 (98%)	1	678/712 (95%)	0.81	493/703 (70%)	0.32	646/677 (95%)	0.9	329/410 (80%)	0.73	394/403 (98%)	0.81
Radio	560/572 (98%)	1	581/613 (95%)	0.38	403/607 (66%)	0.1	561/581 (97%)	0.15	285/341 (84%)	0.09	331/341 (97%)	0.14
Sources of Drinking Water												
Drinking	482/492 (98%)	0.79	501/531 (94%)	0.49	363/528 (69%)	0.58	466/490 (95%)	0.91	246/295 (83%)	0.05	286/293 (98%)	0.52
Piped	173/177 (98%)		177/187 (95%)		124/185 (67%)		179/186 (96%)		73/93 (78%)		92/94 (98%)	
Well	707/724 (98%)		732/760 (96%)		520/755 (69%)		692/722 (96%)		340/419 (81%)		411/418 (98%)	
River	30/30 (100%)		32/33 (97%)		23/31 (74%)		31/31 (100%)		9/14 (64%)		15/15 (100%)	
Rain	5/5 (100%)		5/5 (100%)		3/4 (75%)		3/5 (60%)		1/1 (100%)		1/1 (100%)	
Estuary	19/20 (95%)		21/23 (91%)		18/22 (82%)		18/20 (90%)		8/14 (57%)		12/13 (92%)	
Other	8/8 (100%)		8/8 (100%)		3/7 (43%)		8/8 (100%)		4/7 (57%)		7/7 (100%)	
Pet/farming animal exposure												
Yes	1,299/1,330 (98%)	0.52	1,348/1,411 (96%)	0.4	955/1,398 (68%)	0.2	1,275/1,333 (96%)	0.51	615/764 (80%)	0.65	745/759 (98%)	0.23
No	125/126 (99%)		128/136 (94%)		99/134 (74%)		122/129 (95%)		66/79 (84%)		79/82 (96%)	

Table 3 cont.

Mother	Diphtheria, n/N(%) (n=1,424)	p-value	Tetanus, n/N (%) (n=1,476)	p-value	Pertussis, n/N (%) (n=1,054)	p-value	HIB, n/N (%) (n=1,397)	p-value	Measles, n/N (%) (n=681)	p-value	Rubella, n/N (%) (n=824)	p-value
Race												
Afro-Ecuadorian	335/347 (97%)	**0.01**	347/370 (94%)	0.06	248/369 (67%)	0.7	334/353 (95%)	0.12	141/185 (76%)	0.2	174/180 (97%)	0.27
Mixed	1,085/1,104 (98%)		1,124/1,171 (96%)		802/1,157 (69%)		1,059/1,104 (96%)		537/655 (82%)		647/658 (98%)	
Indigenous	4/5 (80%)		5/6 (83%)		4/6 (67%)		4/5 (80%)		3/3 (100%)		3/3 (100%)	
Civil Status												
Single	154/154 (100%)	**0.04**	154/162 (95%)	0.07	110/159 (69%)	0.05	142/151 (94%)	0.7	76/98 (78%)	0.51	93/98 (95%)	0.17
Married	211/212 (99%)		230/233 (99%)		172/230 (75%)		210/217 (97%)		113/134 (84%)		132/133 (99%)	
Divorced	7/7 (100%)		7/7 (100%)		7/7 (100%)		7/7 (100%)		2/3 (67%)		3/3 (100%)	
Widow	6/6 (100%)		6/6 (100%)		3/6 (50%)		6/6 (100%)		3/3 (100%)		3/3 (100%)	
Other	1,046/1,077 (97%)		1,079/1,139 (95%)		762/1,130 (67%)		1,032/1,081 (95%)		487/605 (80%)		593/604 (98%)	
Education												
Illiterate	19/19 (100%)	0.19	18/20 (90%)	0.16	9/20 (45%)	0.07	18/19 (95%)	0.78	7/11 (64%)	0.39	11/11 (100%)	0.86
Elementary	525/541 (97%)		551/582 (95%)		386/576 (67%)		518/542 (96%)		231/289 (80%)		278/285 (98%)	
Secondary	749/765 (98%)		780/810 (96%)		564/804 (70%)		732/768 (95%)		378/461 (82%)		452/461 (98%)	
Superior	131/131 (100%)		127/135 (94%)		95/132 (72%)		129/133 (97%)		65/82 (79%)		83/84 (99%)	
Smoking during pregnancy												
Yes	1,411/1,442 (98%)	0.27	17/18 (94%)	0.57	12/18 (67%)	0.8	12/13 (92%)	0.45	2/6 (33%)	**0.01**	6/6 (100%)	1
No	13/14 (93%)		1,459/1,529 (95%)		1,042/1,514 (69%)		1,385/1,449 (96%)		679/837 (81%)		818/835 (98%)	
Vaccines administered during pregnancy												
Yes	1,359/1,390 (98%)	1	1,411/1,479 (95%)	1	1,009/1,467 (69%)	1	1,337/1,398 (96%)	0.39	657/808 (81%)	0.08	790/807 (98%)	1
No	64/65 (98%)		64/67 (96%)		44/64 (69%)		59/63 (94%)		24/35 (69%)		34/34 (100%)	
Father												
Civil Status												
Single	92/92 (100%)	0.35	89/94 (95%)	**0.02**	66/91 (73%)	0.12	84/89 (94%)	0.68	41/52 (79%)	0.45	48/52 (92%)	**0.006**
Married	216/219 (99%)		239/242 (99%)		179/239 (75%)		217/224 (97%)		117/137 (85%)		134/135 (99%)	
Divorced	7/7 (100%)		6/7 (86%)		6/8 (75%)		7/7 (100%)		3/4 (75%)		3/4 (75%)	
Widow	2/2 (100%)		2/2 (100%)		2/2 (100%)		2/2 (100%)		0/0 (0%)		0/0 (0%)	
Other	1,050/1,079 (97%)		1,084/1,143 (95%)		760/1,133 (67%)		1,037/1,085 (96%)		489/608 (80%)		597/608 (98%)	
Education												
Illiterate	37/38 (97%)	0.91	38/41 (93%)	0.43	23/41 (56%)	0.16	36/38 (95%)	0.69	13/21 (62%)	0.15	18/21 (86%)	**0.006**
Primary	497/508 (98%)		535/560 (96%)		375/555 (68%)		484/507 (95%)		229/278 (82%)		271/274 (99%)	
Secondary	650/666 (98%)		667/695 (96%)		488/688 (71%)		646/672 (96%)		327/399 (82%)		393/400 (98%)	
Superior	120/122 (98%)		117/125 (94%)		88/122 (72%)		116/123 (94%)		58/73 (79%)		69/72 (96%)	

Table 3 cont.

Child	Diphtheria, n/N(%) (n=1,424)	p-value	Tetanus, n/N (%) (n=1,476)	p-value	Pertussis, n/N (%) (n=1,054)	p-value	HIB, n/N (%) (n=1,397)	p-value	Measles, n/N (%) (n=681)	p-value	Rubella, n/N (%) (n=824)	p-value
Sex												
Male	718/736 (98%)	0.59	740/781 (95%)	0.23	517/772 (67%)	0.12	705/739 (95%)	0.8	339/418 (81%)	0.86	411/420 (98%)	0.81
Female	706/720 (98%)		736/766 (96%)		537/760 (71%)		692/723 (96%)		342/425 (80%)		413/421 (98%)	
Type of birth												
Vaginal	1,057/1,082 (98%)	0.84	1,101/1,158 (95%)	0.39	784/1,146 (68%)	0.75	1,039/1,087 (96%)	0.9	496/628 (79%)	**0.03**	610/624 (98%)	0.58
Cesarean	365/372 (98%)		373/387 (96%)		268/384 (70%)		356/373 (95%)		185/215 (86%)		214/217 (99%)	
Dystrophic	2/2 (100%)		2/2 (100%)		2/2 (100%)		2/2 (100%)		0/0 (0%)		0/0 (0%)	
Experienced wheezing since birth												
Yes	71/75 (95%)	0.15	85/88 (97%)	1	51/88 (58%)	**0.03**	77/79 (97%)	0.57	36/41 (88%)	0.23	43/43 (100%)	0.62
No	989/1,015 (97%)		1,045/1,091 (96%)		748/1,079 (69%)		976/1,023 (95%)		439/558 (79%)		535/551 (97%)	
Antibiotic use since birth												
Yes	394/409 (96%)	**0.03**	435/450 (97%)	0.14	307/447 (69%)	0.95	394/411 (96%)	0.78	174/225 (77%)	0.14	215/221 (97%)	0.41
No	1,030/1,047 (98%)		1,041/1,097 (95%)		747/1,085 (69%)		1,003/1,051 (95%)		507/618 (82%)		609/620 (98%)	
Day care												
Yes	29/29 (100%)	1	29/31 (94%)	0.37	15/30 (50%)	**0.04**	28/30 (93%)	0.39	9/13 (69%)	0.32	13/13 (100%)	1
No	1,034/1,064 (97%)		1,106/1,153 (96%)		788/1,142 (69%)		1,028/1,075 (96%)		467/587 (80%)		566/582 (97%)	
Weight-by-Height z-score												
Severe to Moderate Malnutrition	22/22 (100%)	1	22/23 (96%)	0.56	10/21 (48%)	0.11	20/21 (95%)	0.52	6/8 (75%)	0.92	8/8 (100%)	0.5
Average	679/699 (97%)		724/760 (95%)		521/749 (70%)		679/710 (96%)		309/394 (78%)		377/388 (97%)	
Overweight	30/30 (100%)		33/33 (100%)		23/33 (70%)		31/31 (100%)		11/14 (79%)		14/15 (93%)	

Table 4: Characteristics of the Gemetric Mean Titer (GMT) Vaccine Measurement Following Infant Vaccination for Oral Vaccines

	Rotavirus, no. (n=1,048)	GMT (95% CI)	OPV3, no. (n=1,569)	GMT (95% CI)	OPV1, no. (n=1,596)	GMT (95% CI)
Household & Environment						
SES						
Low	477	33 (29, 38)	456	2,740 (2512, 2988)	411	8,624 (7813, 9520)
Medium to High	1,149	28 (26, 31)	1,093	2,497 (2360, 2643)	971	7,552 (7053, 8085)
Crowding						
Yes	652	32 (29, 36)	616	2,506 (2315, 2713)	548	7,874 (7152, 8669)
No	974	28 (26, 31)	933	2,607 (2458, 2764)	834	7,844 (7323, 8402)
Family Bathroom						
Latrine	1,089	31 (28, 34)	1,041	2,669 (2520, 2827)	938	8,228 (7688, 8807)
Toilet service	513	27 (24, 30)	485	2,342 (2148, 2554)	423	7,017 (6326, 7784)
Field	24	48 (28, 84)	23	2,998 (2362, 3806)	21	9,632 (6907, 13432)
Number of Bedrooms in the House						
1 to 2	1,150	31 (29, 34)	1,096	2,530 (2390, 2677)	985	7,722 (7212, 8269)
3 to 7	476	26 (23, 30)	453	2,657 (2437, 2897)	397	8,197 (7429, 9045)
Electrical Appliances in the Home						
Refrigerator	1,094	28 (26, 31)	1,044	2,508 (2366, 2658)	927	7,559 (7042, 8114)
Television	1,443	29 (27, 45)	1,377	2,541 (2417, 2672)	1,231	7,760 (7307, 8241)
Stereo	750	29 (26, 32)	713	2,627 (2451, 2816)	639	8,155 (7507, 8859)
Radio	650	29 (26, 33)	621	2,468 (2291, 2659)	540	7,393 (6766, 8077)
Sources of Drinking Water						
Drinking	563	32 (28, 36)	534	2,625 (2417, 2851)	482	8,197 (7408, 9071)
Piped	200	29 (24, 35)	190	2,634 (2273, 3053)	159	8,701 (7383, 10254)
Well	793	28 (26, 31)	756	2,532 (2371, 2705)	681	7,562 (7009, 8159)
River	33	28 (16, 49)	33	2,363 (1741, 3207)	29	5,941 (3569, 9892)
Rain	6	51 (9, 298)	5	1,936 (1057, 3548)	3	5,355 (270, 106160)
Estuary	23	35 (19, 63)	23	2,208 (1551, 3144)	20	6,741 (4182, 10867)
Other	8	46 (6, 347)	8	2,792 (1124, 6936)	8	9,486 (3882, 23180)
Pet/farming animal exposure						
Yes	1,483	30 (28, 32)	1,413	2,552 (2428, 2682)	1,260	7,722 (7275, 8196)
No	143	30 (24, 37)	136	2,721 (2322, 3189)	122	9,387 (7965, 11062)

Table 4 cont.

	Rotavirus, no. (n=1,048)	GMT (95% CI)	OPV3, no. (n=1,569)	GMT (95% CI)	OPV1, no. (n=1,596)	GMT (95% CI)
Mother						
Race						
Afro-Ecuadorian	394	35 (30, 41)	378	2,918 (2648, 3216)	340	9,225 (8257, 10307)
Mixed	1,226	28 (26, 31)	1,165	2,459 (2329, 2597)	1,036	7,462 (6990, 7966)
Indigenous	6	20 (10, 43)	6	3,035 (1736, 5306)	6	6,267 (2234, 17583)
Civil Status						
Single	175	32 (26, 40)	163	2,393 (2051, 2792)	147	7,334 (6099, 8818)
Married	244	27 (23, 32)	234	2,603 (2302, 2945)	204	7,564 (6538, 8751)
Divorced	8	36 (10, 133)	7	2,676 (770, 9306)	6	13,935 (4706, 41258)
Widow	7	39 (15, 103)	6	3,029 (1189, 7717)	6	8,727 (3413, 22314)
Other	1,192	30 (28, 32)	1,139	2,582 (2444, 2727)	1,019	7,963 (7460, 8499)
Education						
Illiterate	23	36 (21, 62)	21	3,727 (2459, 5649)	18	10,938 (6792, 17613)
Elementary	605	31 (28, 35)	578	2,576 (2387, 2780)	522	7,953 (7270, 8699)
Secondary	854	29 (26, 31)	810	2,564 (2400, 2740)	715	7,905 (7316, 8541)
Superior	144	30 (24, 37)	140	2,401 (2047, 2816)	127	6,883 (5547, 8540)
Smoking during pregnancy						
Yes	22	37 (20, 70)	19	3,144 (1814, 5449)	17	10,939 (6307, 18973)
No	1,604	30 (28, 32)	1,530	2,560 (2441, 2684)	1,365	7,824 (7393, 8279)
Vaccines administered during pregnancy						
Yes	1,557	30 (28, 32)	1,482	2,549 (2429, 2676)	1,319	7,769 (7332, 8232)
No	68	32 (23, 45)	66	2,984 (2392, 3721)	62	9,910 (7824, 12552)
Father						
Civil Status						
Single	103	30 (23, 40)	94	2,356 (1927, 2879)	86	7,142 (5727, 8906)
Married	254	28 (23, 33)	241	2,645 (2345, 2982)	212	7,817 (6777, 9017)
Divorced	9	35 (21, 62)	8	3,580 (1722, 7443)	7	11,449 (4506, 29094)
Widow	2	68 (-,-)	2	1,284 (10, 157219)	2	6,817 (20, 2268333)
Other	1,197	30 (28, 33)	1,145	2,566 (2429, 2712)	1,021	7,910 (7408, 8447)
Education						
Illiterate	44	40 (27, 59)	44	3,317 (2640, 4167)	38	11,891 (9549, 14808)
Primary	587	31 (27, 34)	558	2,547 (2351, 2760)	497	7,647 (6959, 8404)
Secondary	727	29 (26, 32)	694	2,548 (2371, 2738)	612	7,869 (7214, 8583)
Superior	135	25 (20, 31)	128	2,512 (2159, 2922)	119	7,597 (6481, 8906)

Table 4 cont.

Child	Rotavirus, no. (n=1,048)	GMT (95% CI)	OPV3, no. (n=1,569)	GMT (95% CI)	OPV1, no. (n=1,596)	GMT (95% CI)
Sex						
Male	822	31 (28, 34)	773	2,543 (2374, 2724)	692	8,078 (7450, 8759)
Female	804	29 (26, 32)	776	2,590 (2426, 2764)	690	7,640 (7064, 8262)
Type of birth						
Vaginal	1,216	30 (28, 32)	1,159	2,614 (2473, 2763)	1,031	8,185 (7674, 8729)
Cesarean	408	29 (25, 33)	388	2,442 (2229, 2674)	349	7,007 (6245, 7863)
Dystrophic	2	27 (0, <1000)	2	939 (48, 18359)	2	2,351 (4, 1281257)
Experienced wheezing since birth						
Yes	93	38 (29, 50)	89	2,498 (2008, 3107)	70	9,066 (6894, 11922)
No	1,137	30 (28, 33)	1,087	2,596 (2453, 2747)	969	7,720 (7212, 8264)
Antibiotic use since birth						
Yes	467	32 (28, 36)	448	2,539 (2311, 2789)	401	8,107 (7270, 9040)
No	1,159	29 (27, 31)	1,101	2,578 (2440, 2722)	981	7,756 (7263, 8282)
Day care						
Yes	36	54 (33, 89)	31	3,555 (2456, 5145)	27	12,898 (8981, 18523)
No	1,200	30 (28, 33)	1,150	2,576 (2437, 2722)	1,017	7,730 (7229, 8266)
Weight-to-Height z-score						
Severe to Moderate Malnutrition	23	33 (17, 62)	23	2,756 (1687, 4504)	22	9,995 (7063, 14145)
Average	779	30 (27, 34)	758	2,606 (2438, 2786)	682	7,669 (7074, 8314)
Overweight	34	21 (13, 25)	32	1,727 (1266, 2357)	28	4,033 (2333, 6972)

Table 5: Characteristics of the Geometric Mean Titer (GMT) Vaccine Measurement Following Infant Vaccination for Intramuscular Vaccines

	Diphtheria, no. (n=1,593)	GMT (95% CI)	Tetanus, no. (n=1,593)	GMT (95% CI)	Pertussis, no. (n=1,593)	GMT (95% CI)	HIB, no. (n=1,593)	GMT (95% CI)	Measles, no. (n=936)	GMT (95% CI)	Rubella, no. (n=936)	GMT (95% CI)
Household & Environment												
SES												
Low	425	138 (122, 156)	466	1,076 (965, 1199)	466	13 (11, 15)	465	4,240 (3629, 4955)	223	543 (431, 684)	223	89 (79, 99)
Medium to High	1,059	131 (121, 142)	1,117	1,011 (937, 1,090)	1,117	13 (12, 15)	1,118	3,612 (3266, 3995)	644	651 (567, 746)	644	93 (87, 99)
Crowding												
Yes	588	142 (128, 157)	633	1,090 (987, 1204)	633	14 (12, 16)	632	4,129 (3610, 4721)	310	658 (540, 801)	310	97 (89, 107)
No	896	128 (117, 139)	950	991 (915, 1,073)	950	13 (11, 14)	951	3,574 (3205, 3986)	557	601 (520, 696)	557	89 (83, 96)
Family Bathroom												
Latrine	987	132 (122, 143)	1,062	1,012 (938, 1092)	1,062	14 (12, 15)	1,063	3,743 (3374, 4151)	558	628 (542, 729)	558	90 (84, 95)
Toilet service	476	136 (122, 152)	497	1,071 (959, 11996)	497	12 (10, 14)	496	3,854 (3315, 4481)	298	635 (522, 773)	298	97 (88, 107)
Field	21	111 (57, 218)	24	968 (555, 1686)	24	9 (4, 20)	24	4,380 (2183, 8787)	11	187 (85, 411)	11	62 (38, 100)
Number of Bedrooms in the House												
1 to 2	1,048	133 (123, 144)	1,121	1,037 (963, 1118)	1,121	13 (11, 14)	1,121	3,787 (3423, 4189)	591	620 (539, 713)	591	93 (87, 99)
3 to 7	436	133 (117, 150)	462	1,010 (902, 1130)	462	14 (12, 16)	462	3,785 (3240, 4422)	276	624 (503, 774)	276	90 (81, 100)
Electrical Appliances in the Home												
Refrigerator	1,003	134 (124, 145)	1,064	1,038 (963, 1119)	1,064	13 (12, 15)	1,065	3,558 (3213, 3940)	610	641 (557, 738)	610	91 (85, 98)
Television	1,330	133 (124, 143)	1,407	1,027 (961, 1258)	1,407	13 (12, 15)	1,408	3,692 (3375, 4039)	783	640 (565, 724)	783	93 (87, 98)
Stereo	693	131 (119, 144)	731	1,008 (919, 1106)	731	14 (12, 16)	731	3,539 (3127, 4006)	423	639 (538, 759)	423	92 (85, 99)
Radio	586	131 (118, 146)	629	1,024 (926, 1133)	629	12 (10, 13)	630	3,969 (3470, 4540)	354	643 (540, 767)	354	92 (84, 101)
Sources of Drinking Water												
Drinking	507	118 (105, 132)	548	959 (861, 1068)	548	14 (12, 16)	548	3,545 (3062, 4104)	308	676 (561, 814)	308	86 (78, 95)
Piped	182	138 (114, 167)	192	1,010 (838, 1218)	192	13 (10, 17)	192	3,405 (2687, 4316)	95	482 (337, 691)	95	113 (95, 135)
Well	731	143 (130, 157)	774	1,061 (973, 1157)	774	13 (11, 14)	774	3,991 (3546, 4493)	427	648 (547, 767)	427	94 (86, 101)
River	31	158 (101, 246)	33	1,616 (1059, 2465)	33	16 (8, 32)	33	7,246 (3990, 13160)	15	478 (125, 1825)	15	104 (62, 173)
Rain	5	125 (53, 290)	5	1,182 (441, 3171)	5	8 (1, 51)	5	306 (65, 1435)	1	563 (-, -)	1	104 (-,-)
Estuary	20	138 (72, 266)	23	1,123 (584, 2159)	23	20 (10, 40)	23	4,474 (1698, 11793)	14	334 (74, 1509)	14	61 (32, 115)
Other	8	103 (25, 413)	8	1,272 (536, 3,014)	8	4 (1, 11)	8	5,454 (1037, 28684)	7	215 (45, 1030)	7	63 (31, 172
Pet/farming animal exposure												
Yes	1,354	133 (124, 143)	1,443	1,027 (962, 1095)	1,443	13 (12, 14)	1,443	3,765 (3446, 4114)	784	620 (547, 702)	784	91 (85, 96)
No	130	133 (107, 164)	140	1,060 (845, 1329)	140	16 (12, 21)	140	4,011 (3020, 5327)	83	634 (445, 902)	83	104 (86, 125)

Table 5 cont.

Mother	Diphtheria, no. (n=1,593)	GMT (95% CI)	Tetanus, no. (n=1,593)	GMT (95% CI)	Pertussis, no. (n=1,593)	GMT (95% CI)	HIB, no. (n=1,593)	GMT (95% CI)	Measles, no. (n=936)	GMT (95% CI)	Rubella, no. (n=936)	GMT (95% CI)
Race												
Afro-Ecuadorian	357	120 (104, 137)	383	868 (763, 988)	383	12 (10, 14)	383	3,314 (2789, 3937)	190	496 (385, 639)	190	84 (74, 96)
Mixed	1,121	139 (129, 150)	1,194	1,096 (1021, 1176)	1,194	14 (12, 15)	1,194	4,002 (3632, 4409)	674	663 (581, 758)	674	94 (89, 101)
Indigenous	6	27 (7, 100)	6	225 (91, 559)	6	6 (1, 36)	6	307 (123, 767)	3	348 (169, 717)	3	37 (24, 56)
Civil Status												
Single	157	144 (118, 142)	164	1,063 (867, 1304)	164	12 (9, 17)	164	3,549 (2690, 4683)	99	532 (373, 758)	99	91 (76, 109)
Married	220	147 (124, 175)	239	1,248 (1080, 1441)	239	18 (14, 23)	240	4,167 (3392, 5120)	140	662 (504, 870)	140	100 (87, 115)
Divorced	7	144 (64, 322)	7	804 (388, 1666)	7	31 (13, 76)	7	2,849 (954, 8506)	3	237 (1, 70884)	3	79 (10, 645)
Widow	6	283 (103, 780)	6	1,476 (455, 4795)	6	13 (1, 157)	6	6,307 (1163, 34209)	3	942 (262, 3385)	3	52 (16, 170)
Other	1,094	128 (119, 139)	1,167	985 (915, 1060)	1,167	12 (11, 14)	1,166	3,743 (3389, 4133)	622	629 (546, 724)	622	90 (85, 97)
Education												
Illiterate	20	83 (41, 169)	21	528 (303, 922)	21	6 (2, 13)	21	2,259 (1043, 4891)	11	356 (82, 1541)	11	73 (40, 132)
Elementary	554	145 (127, 165)	592	1,078 (971, 1197)	592	12 (10, 14)	591	3,834 (3333, 4410)	295	596 (484, 735)	295	88 (80, 98)
Secondary	776	136 (118, 158)	829	999 (919, 1086)	829	14 (12, 16)	830	3,630 (3230, 4079)	473	629 (540, 733)	473	94 (87, 101)
Superior	134	220 (111, 434)	141	1,118 (899, 1390)	141	15 (11, 20)	141	4,977 (3790, 6536)	88	711 (476, 1060)	88	98 (82, 117)
Smoking during pregnancy												
Yes	15	146 (63, 340)	19	1,098 (556, 2168)	19	11 (5, 25)	19	2,067 (908, 4705)	7	113 (34, 380)	7	68 (34, 134)
No	1,469	133 (124, 142)	1,564	1029 (966, 1095)	1,564	13 (12, 14)	1,564	3,814 (3503, 4153)	860	630 (560, 708)	860	92 (87, 98)
Vaccines administered during pregnancy												
Yes	1,417	132 (124, 142)	1,514	1,036 (972, 1104)	1,514	13 (12, 14)	1,514	3,826 (3509, 4170)	832	631 (560, 711)	832	92 (87, 98)
No	66	151 (112, 204)	68	894 (662, 1205)	68	12 (8, 18)	68	3,062 (1976, 4746)	35	424 (212, 847)	35	83 (63, 109)
Father												
Civil Status												
Single	93	156 (121, 200)	95	1,126 (858, 1476)	95	16 (11, 23)	95	4,054 (2804, 5861)	52	523 (315, 869)	52	86 (67, 111)
Married	227	144 (122, 172)	248	1,233 (1073, 1417)	248	18 (14, 22)	249	4,086 (3338, 5002)	143	687 (525, 899)	143	100 (87, 115)
Divorced	7	99 (55, 177)	8	518 (139, 1923)	8	23 (5, 111)	8	2,566 (763, 8631)	4	224 (13, 3837)	4	46 (10, 209)
Widow	2	66 (0, <1000)	2	686 (92, 5130)	2	15 (0, 582)	2	2,404 (15, 392797)	0	N/A	0	N/A
Other	1,097	129 (119, 139)	1,171	991 (921, 1066)	1,171	12 (11, 13)	1,170	3,797 (3440, 4192)	625	630 (548, 725)	625	91 (85, 97)
Education												
Illiterate	40	118 (78, 178)	43	782 (508, 1202)	43	7 (4, 12)	43	4,430 (2735, 7177)	22	279 (112, 694)	22	61 (38, 99)
Primary	518	134 (120, 150)	572	1,064 (960, 1180)	572	13 (11, 15)	571	3,758 (3262, 4330)	285	638 (522, 778)	285	91 (82, 100)
Secondary	678	136 (124, 150)	711	1,013 (925, 1109)	711	14 (12, 16)	712	3,812 (3360, 4326)	410	668 (564, 792)	410	96 (89, 105)
Superior	123	119 (96, 149)	130	1,042 (820, 1324)	130	15 (11, 20)	130	4,048 (3013, 5438)	75	566 (371, 863)	75	87 (71, 107)

Table 5 cont.

Child	Diphtheria, no. (n=1,593)	GMT (95% CI)	Tetanus, no. (n=1,593)	GMT (95% CI)	Pertussis, no. (n=1,593)	GMT (95% CI)	HIB, no. (n=1,593)	GMT (95% CI)	Measles, no. (n=936)	GMT (95% CI)	Rubella, no. (n=936)	GMT (95% CI)
Sex												
Male	750	131 (120, 144)	798	1,023 (935, 1119)	798	12 (11, 14)	797	3,527 (3132, 3973)	432	618 (524, 729)	432	88 (81, 96)
Female	734	135 (123, 149)	785	1,036 (951, 1130)	785	14 (12, 16)	786	4,068 (3607, 4588)	435	624 (528, 738)	435	95 (88, 103)
Type of birth												
Vaginal	1,105	129 (120, 139)	1,182	1,012 (941, 1088)	1,182	13 (11, 14)	1,181	3,632 (3289, 4010)	644	567 (494, 651)	644	88 (82, 94)
Cesarean	377	145 (127, 165)	399	1,084 (960, 1224)	399	14 (12, 17)	400	4,280 (3640, 5032)	223	807 (650, 1004)	223	105 (94, 116)
Dystrophic	2	255 (0, <1000)	2	817 (168, 3971)	2	17 (0, <1000)	2	4,099 (0, 1.66E11)	0	N/A	0	N/A
Experienced wheezing since birth												
Yes	75	100 (71, 142)	89	1,005 (764, 1320)	89	9 (6, 13)	89	3,928 (2688, 5739)	43	797 (500, 1271)	43	90 (70, 116)
No	1,036	132 (122, 143)	1,114	1,044 (970, 1122)	1,114	13 (12, 15)	1,113	3,692 (3336, 4086)	569	515 (445, 596)	569	85 (79, 91)
Antibiotic use since birth												
Yes	413	127 (112, 145)	456	1,042 (933, 1163)	456	14 (11, 16)	456	3,453 (2964, 4023)	227	514 (404, 653)	227	81 (73, 91)
No	1,071	135 (125, 146)	1,127	1,025 (950, 1105)	1,127	13 (12, 14)	1,127	3,930 (3551, 4350)	640	664 (581, 760)	640	96 (90, 102)
Day care												
Yes	29	160 (114, 225)	31	1,038 (663, 1627)	31	5 (3, 9)	31	2,660 (1272, 5563)	13	457 (135, 1542)	13	108 (64, 184)
No	1,085	129 (119, 139)	1,177	1,043 (971, 1120)	1,177	13 (12, 15)	1,176	3,744 (3394, 4131)	600	533 (463, 613)	600	85 (79, 91)
Weight-to-Height z-score												
Severe to Moderate Malnutrition	22	192 (109, 335)	23	986 (557, 1746)	23	6 (3, 13)	23	4,768 (2120, 10725)	8	326 (99, 1075)	8	62 (31, 125)
Average	713	124 (113, 137)	771	1,041 (928, 1109)	771	13 (12, 15)	771	3,428 (3039, 3867)	403	473 (399, 560)	403	82 (76, 90)
Overweight	30	190 (131, 277)	33	1,946 (1408, 2691)	33	13 (6, 28)	33	7,709 (4697, 12654)	15	529 (183, 1532)	15	89 (50, 158)

Table 6: Effects of Socioeconomic and Environmental Exposures on Achievement of a Designated Protective Antibody Threshold Following Infant Vaccination

	Rotavirus, OR (95% CI)	OPV3, OR (95% CI)	Tetanus, OR (95% CI)	Pertussis, OR (95% CI)	HIB, OR (95% CI)	Measles, OR (95% CI)
Maternal Education						
Illiterate	0.85 (0.32, 2.23)					
Primary	0.73 (0.48, 1.09)					
Secondary	0.69 (0.47, 1.02)					
Superior	Referent					
Household Crowding						
Yes	1.24 (0.99, 1.55)				1.30 (0.77, 2.19)	
No	Referent				Referent	
Socioeconomic Status						
Low	0.81 (0.64, 1.03)		0.62 (0.35, 1.11)			
Medium to High	Referent		Referent			
Maternal Vaccination during Pregnancy						
Yes		0.80 (0.47, 1.39)	1.0 (0.31, 3.28)			1.61 (0.74, 3.51)
No		Referent	Referent			Referent
Antibiotic Use Since Birth						
Yes			1.55 (0.87, 2.77)	0.99 (0.78, 1.26)		
No			Referent	Referent		
Child's Sex						
Male				1.19 (0.96, 1.48)		
Female				Referent		
Pet/farming Animal Exposure						
Yes				1.31 (0.88, 1.96)		
No				Referent		
Sources of Drinking Water						
Drinking						3.58 (0.77, 16.62)
Piped						2.58 (0.53, 12.60)
Well						3.07 (0.67, 14.10)
River						1.35 (0.21, 8.69)
Rain						N/A
Estuary						1.11 (0.18, 7.06)
Other						Referent

Table 7: Effects of Socioeconomic and Environmental Exposures on the Gemetric Mean Titer Vaccine Measurement Following Infant Vaccination

	Rotavirus GMT Ratio (95% Cl)	OPV3 GMT Ratio (95% CI)	OPV1 GMT Ratio (95% CI)	Diphtheria GMT Ratio (95% CI)	Tetanus GMT Ratio (95% CI)	Pertussis GMT Ratio (95% CI)	HIB GMT Ratio (95% CI)	Mealses GMT Ratio (95% CI)
Maternal Education								
Illiterate							**-0.89 (-1.68, -0.10)**	
Primary							-0.32 (-0.64, 0.003)	
Secondary							**-0.34 (-0.65, -0.03)**	
Superior							Referent	
Household Crowding								
Yes		-0.06 (-0.16, 0.03)					0.18 (-0.001, 0.35)	
No		Referent					Referent	
Socioeconomic Status								
Low	**-0.16 (-0.31, -0.01)**	-0.10 (-0.21, 0.003)						
Medium to High	Referent	Referent						
Maternal Vaccination during Pregnancy								
Yes		-0.15 (-0.38, 0.09)	-0.24 (-0.51, 0.03)					0.40 (-0.20, 0.99)
No		Referent	Referent					Referent
Antibiotic Use Since Birth								
Yes	0.09 (-0.06, 0.24)		0.04 (-0.08, 0.17)		0.02 (-0.12, 0.16)	0.05 (-0.16, 0.25)		
No	Referent		Referent		Referent	Referent		
Sex								
Male						0.13 (-0.05, 0.32)		
Female						Referent		
Pet/farming Animal Exposure								
Yes			0.19 (-0.01, 0.39)	0.02 (-0.22, 0.25)		0.16 (-0.17, 0.48)		
No			Referent	Referent		Referent		
Sources of Drinking Water								
Drinking				0.14 (-0.77, 1.04)	-0.27 (-1.16, 0.61)			
Piped				0.30 (-0.62, 1.21)	-0.22 (-1.12, 0.67)			
Well				0.33 (-0.57, 1.24)	-0.17 (-1.05, 0.71)			
River				0.43 (-0.57, 1.44)	0.25 (-0.73, 1.22)			
Rain				0.20 (-1.25, 1.64)	-0.06 (-1.47, 1.35)			
Estuary				0.30 (-0.76, 1.36)	-0.12 (-1.13, 0.90)			
Other				Referent	Referent			

Discussion

Differences in which predictors were included in vaccine models were seen in both outcome measurements. Even further, some vaccines differed in predictors between outcome measurement types. For example, in the rotavirus seropositivity model, maternal education, household crowding, and socioeconomic status were included in the model (Table 6). But in the rotavirus GMT model, only socioeconomic status and child's use of antibiotics since birth were included in the model (Table 7). This phenomenon was seen across many of the vaccines with the exception of pertussis. It was expected that oral vaccines would be more similar to one another than to intramuscular vaccines and this was not observed. All vaccine models were relatively different from one another. The majority of the models in this analysis did not provide statistically significant results, demonstrating that the predictors included in the model were not adequate in describing the odds of reaching seropositivity or in describing the association with GMTs. However, socioeconomic status was found to be a significant predictor for the rotavirus GMT model.

The results indicate that as SES increases, the likelihood of having a higher GMT decreases (-0.16, 95% CI: -0.31, -0.01). This result is contrary to what previous research would indicate, where lower socioeconomic status is considered a risk factor for decreased oral vaccine response (7). There are multiple potential reasons for this result. Because antibodies generated from rotavirus vaccine and natural exposure cannot be distinguished from one another, it is possible that children who are lower SES could have more natural exposures, resulting in higher antibody titers being detected. Alternatively, socioeconomic status could be acting as a proxy for other potentially relevant factors that

may impact vaccine response that is causing the association seen in the analysis. For example, there could be a factor related to water, sanitation, and hygiene that is more prominently seen in lower socioeconomic populations than in middle to higher settings that is truly what is causing the association between low socioeconomic status and increased GMT measurements. Further analysis into the socioeconomic status variable would need to be performed in order to validate this explanation but it is possible due to previous research that has demonstrated the numerous factors that are associated with socioeconomic status as well as vaccine response (5, 7, 13, 24, 28).

Previous research has also demonstrated that intramuscular vaccines usually do not differ in response between individual characteristics or different environments such as developing versus developed countries (34). However, this analysis resulted in maternal education at an illiterate level (-0.89, 95% CI: -1.68, -0.10) and a secondary level (-0.34, 95% CI: -0.65, -0.03) as a significant predictor in the HIB vaccine model, indicating that maternal education may play a role in increasing vaccine response. These results suggest that having a maternal education level lower than some amount of higher education increases the likelihood of a higher antibody titer. These results were not expected and can potentially be explained by a confounding factor that is impacting both maternal education and vaccine response that is unaccounted for in this analysis.

There are several strengths in this study. The data in this analysis originated from a prospective cohort study, resulting in a clear temporal sequence between potential predictors and vaccine response. The study design also allowed for the examination of multiple predictors, of which there were dozens in this analysis. Because there were also multiple vaccines with two different outcomes, this allowed for several comparisons.

This study was an extensive exploratory analysis that examined the relationships between exposures and vaccine measurement variables very closely. A thorough literature review was also performed prior to starting the analysis that guided the exploratory analysis in a way that was influenced by prior research. Because of these aspects of the project, it was a comprehensive analysis that covered a large scope of information.

However, there are also several limitations in this study. Because this was a secondary data analysis, the data was not collected with this study question in mind, resulting in potential variables of interest being unavailable for the analysis. Variables such as water contamination, maternal diet, and if possible, child's stool composition would be of interest to better understand how environmental microbial burden impacts oral vaccine response (20). There also was not data for serology or GMT measurements prior to vaccination, calling into question how much of an effect natural exposure had on these measurements in the study and how much of the impact can be attributed solely to vaccination, especially since natural versus rotavirus vaccine responses cannot be discriminated from one another.

In order to better understand how socioeconomic and environmental exposures impact vaccine response, future research should focus on factors such as gut microbiota composition, water contamination, gastrointestinal illness, as well as how nutrition impacts immune responses and subsequently vaccine response. These factors are especially important in understanding oral vaccine response, in which the composition of the gut mucosa is believed to play a large role in decreased vaccine response (5). Socioeconomic and environmental factors are potentially largely intertwined with the composition of the gut mucosa and are therefore important to study and analyze to better

understand vaccine response and subsequently work to improve vaccine response in impacted populations.

Conclusion

This study has demonstrated how broader socioeconomic and environmental factors may play a role in vaccine response in young children. Factors such as socioeconomic status, maternal education, household crowding and other environmental exposures should continue to be considered when analyzing differences in vaccine response between populations. While proximal factors that directly impact the biological mechanisms of vaccine response, especially oral vaccine response, should always be considered, paying attention to population-level characteristics can help to begin to understand why developing or low-income nations still struggle with effective vaccines after years of improved vaccine efficacy and coverage in these populations.

Chapter Three: Public Health Impact

Identifying what factors impact vaccine response, especially oral vaccine response, is a complex area of study. Many different factors such as SES, malnutrition, unsanitary latrine use, and concurrent parasitic infections have been shown to in some way impact vaccine response (5, 7, 13, 24, 28). However, the degree to which each factor impacts vaccine response and whether or not each factor acts more as a proxy for other variables, such as antibodies in a child's blood, a child's nutritional status and breast feeding duration, is difficult to tease apart in statistical analysis and requires further research to better understand their true effect (33, 12, 35). This study demonstrated that broader socioeconomic and environmental factors such as SES may help to predict vaccine response in young child living in Ecuador. In the future, a deeper analysis of what element of SES specifically predicts vaccine response should be studied. It would also be important to better understand the biological mechanisms that broader societal factors, such as low SES, act through to impact vaccine response, particularly for oral vaccines.

Understanding why oral vaccines have been shown to work differently in developed versus developing countries should continue to be an important and crucial goal for public health scientists (1-4). Oftentimes, oral vaccines are needed the most in places where they have been shown to have lower effectiveness (1). In order to continue to improve the health of children worldwide, continued research should be focused on understanding why this occurs and subsequently working to eliminate this as an issue by improving oral vaccines. This study has added to the body of research regarding factors that impact vaccine response and has demonstrated how population-level factors such as

socioeconomic status may play a role in decreasing vaccine response in young children in a developing country.

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