

**SCHOOL OF PUBLIC HEALTH
COLLEGE OF HEALTH SCIENCES
UNIVERSITY OF GHANA**

**ROTAVIRUS DIARRHEA REINFECTION AND ITS OUTCOME ON
WEIGHT AND HOSPITALIZATION – DURATION AMONG
CHILDREN IN GHANA: A RANDOMIZED, DOUBLE – BLINDED,
PLACEBO – CONTROLLED TRIAL**



CLEMENT TETTEH NARH

**THIS THESIS/DISSERTATION IS SUBMITTED TO THE
UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILMENT OF
THE REQUIREMENT FOR THE AWARD OF A MASTER OF
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DECLARATION

I, Clement Tetteh Narh declare that except for the other people's investigations which have duly been acknowledged in this dissertation. This work is the result of my own original research carried out for the award of a Master of Science (Clinical Trials) degree. This dissertation has not been presented elsewhere either in whole or in part for another degree.

Author

.....
Clement Tetteh Narh
(MSc. Clinical Trial Resident – School of Public Health, Legon)

The crest of the University of Ghana is a shield-shaped emblem. The top section is blue with three golden stalks of grain. The bottom section is white with a golden interlocking knot design. Below the shield is a golden ribbon with the Latin motto "INTEGRI PROCEDAMUS" in blue capital letters.

Supervisor

.....
Prof. George E. Armah
(Senior Faculty Member – School of Public Health and
Noguchi Memorial Institute for Medical Research)

DEDICATION

This dissertation is dedicated to my dear wife Naa Merley and the clinical trials baby, Kea Badjo Narh.



ACKNOWLEDGMENT

I foremost thank God for his mercies.

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To you all who may get the opportunity to read this thesis or may not, I extend my best wishes to you for all your support as I count my blessings and name them one by one!

May the good Lord richly reward you!

ABSTRACT

Background

Rotavirus gastroenteritis is a major contributor to the overall burden of diarrhea disease in Africa. Close to 40% of hospital admissions are as a result of diarrhea in children below the ages of five years are traceable to rotavirus. There is incomplete immunity after infection; however repeated infections tend to be less severe than the severity of the first rotavirus infection.

Methods

The study estimated the effect of repeated episodes of the number of severe rotavirus diarrhea on the weight of child at study entry, or hospitalization – duration using proportions and regression models. Univariate and bivariate linear and logistic regression models were used in testing for an association of the repeated episodes of the number of severe rotavirus diarrhea and confounding variables on the weight of child at study entry, or hospitalization – duration as outcomes.

Results

1098 children were randomly assigned to the Vaccine arm and 1102 children to the Placebo arm. Of the three age groups studied, almost 45% of the children in both arms were in the age group 7 – 9 weeks. A cumulative total of 142 and 151 severe diarrhea episodes were recorded in the Vaccine and Placebo arms respectively. However there were only 30 repeated episodes of gastroenteritis recorded. Of these were 16 cases in the vaccine arm and 14 in the control arm. The occurrence of repeated gastroenteritis was

also related to the age at recruitment and was common in the younger age group. Children in the age group 7 – 9 weeks suffered weight loss and hospitalization during the first severe diarrhea episode (Vaccine 66/142 and Placebo 69/151). Fifty-four percent of all children who had diarrhea were hospitalized and 46.0% were outpatients in the Vaccine arm and in the Placebo arm inpatients were 59.7% and outpatients were 40.3%. .

The predominant G genotype of rotaviruses identified in diarrhea stools was G2 (Vaccine 46.5% and Placebo 45.0%).

Children randomized between 7 – 9 weeks and age group 10 – 12 weeks of age were observed to have greater reduction in weight than the younger age groups (P-Value=0.012 and P-value <0.001 respectively).

After adjusting for age group, number of severe diarrhea episode showed a statistical evidence (P-value <0.001) of an association with average weight change in age group 7 – 9 weeks and 10 – 12 weeks in the Vaccine and Placebo arms. The regression coefficient for age group 7 – 9 weeks changed from -0.4 (95% CI=-0.32, 0.24) to -0.08 (95% CI=-0.31, 0.15) in the Placebo arm. Further indicating that vaccination at an earlier age protects against severe diarrhea.

Conclusion

The risk of getting severe diarrhea episode is proportional to age, therefore if children are vaccinated at an early age, the lesser the risks of an infection. There was no link between G genotype and occurrence of repeat rotavirus diarrhea episodes. There was progression in hospitalization with increase in the number severe rotavirus diarrhea episodes.

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LIST OF ACRONYMS

CRF	Case Report Form
EPI	Expanded Programme on Immunization
EU	European Union
KND	Kassena – Nankana District
NMIMR	Noguchi Memorial Institute for Medical Research
USA	United States of America
WHO	World Health Organization
95%CI	95% Confidence Interval
OR	Odds Ratio
LR	Likelihood Ratio

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Diarrhea is a major cause of morbidity and mortality in young children, causing approximately 3 million deaths per year.^{1, 2} About 20% of these diarrhea episodes are caused by rotavirus.³ Rotaviruses are double stranded RNA viruses and members of the Reoviridae with a genome consisting of 11 segments of dsRNA.⁴ Rotaviruses are triple-layered particles with the middle and outer layers comprising of viral proteins VP6, VP7 and VP4. They are classified into groups, subgroups according to the characteristics of the VP6 antigen and genotypes by the VP7 and VP4.^{5, 6}

Rotavirus infection is common worldwide but 80% of all mortality occurs in the developing countries with Africa and Asia.⁷ In Ghana, rotavirus infection is responsible for 39% to 58.9% of diarrheal cases in children.^{5, 8, 9} Rotaviruses are transmitted through the fecal-oral route, but some studies have reported evidence for possible respiratory transmission.¹⁰ Rotavirus illness follows an incubation period of 24 to 48 hours followed by a progression from asymptomatic to severe diarrhea.¹¹ Vomiting, fever, mild to severe dehydration, abdominal pain and respiratory disturbances are the clinical symptoms which usually accompany the watery diarrhea.¹²

Studies that have been conducted to determine the distribution of rotavirus G and P types circulating on the African continent indicated both diverse population of co – circulating types and co – infection with more than one type. Steele in 2000 found out that Rotavirus

G1P[8] was the most common strain found.^{14, 13} Furthermore, the detection of different G and P types indicated that novel viruses may have originated through re – assortment in the study area.

Due to the large effect of morbidity and mortality associated rotavirus infection in children, the WHO recommends the inclusion of rotavirus vaccination of infants alongside improved hygiene and sanitation, and oral rehydration therapy in Ghana's immunization programmes.

Close to 40% of hospital admissions in children below the ages of five years are due to diarrhea caused by rotavirus.^{15, 23} Although repeated infections tend to be less severe than the first rotavirus infection.

In a recent prospective study by Armah et al in 2010 of children with group A Rotavirus infections and reinfections, no evidence of reinfection by group A rotavirus were found.¹⁵ This finding was in contrast with a earlier studies that recorded variable levels of rotavirus reinfection.⁵¹⁻⁵⁷

1.2 Rationale of the Study

The predominance of rotaviruses in causing morbidity and mortality in children is a major issue for healthcare providers and parents. Although hand washing and improved sanitation has helped reduce the incidence of diarrhea in children, notably those attributable to bacteria and parasites diarrhea attributable to rotaviruses viruses has not been reduced. Presently the only intervention available is by vaccination. As part of global efforts to find a rotavirus vaccine, RotaTaq vaccine been licensed and is being used in the United States of America (USA). It was tested in Ghanaian children to determine its Efficacy, Safety, and Immunogenicity between 2006 and 2009.¹⁵ Whilst the study looked at vaccine safety, efficacy and immunogenicity; data was also collected on the circulating strain types, number of re-infections and co-infections in addition to other information.

This study was a secondary analysis of a trial data to determine the number of diarrhea reinfections attributable to rotavirus and the presence or absence to strain types. The study will find out the effect of the number of rotavirus diarrhea reinfection on growth of child and the hospital - duration (as a proxy severity) for diarrhea. Finally, the study estimated and determined confounding variables associated with any of these outcome variables, thus average weight change and hospital – duration of the children as they entered into the trial.

1.3 Statement of the problem

Considering the impact of rotaviruses in causing morbidity and mortality in children, it was necessary to determine if there is any relation between the number of diarrhea infection and the risk factors such as rotavirus genotypes, age and sex of the children with the average weight change of the children and severity of infection.

1.4 Study Objectives

1.4.1 General Objective

To estimate rotavirus diarrhea reinfection and determine its outcome on weight of child at study entry and hospitalization during the first 106 weeks of life - duration among children in the Kassena Nankana District of Ghana.

1.4.2 Specific Objectives

1. To estimate rotavirus diarrhea reinfection among children in Ghana.
2. To determine rotavirus genotypes that causes severe diarrhea reinfection requiring hospitalizations among children in the Kassena Nankana District of Ghana.
3. Determine and Compare outcomes (average weight change of child in the study and Hospital – Duration) of Rotavirus Diarrhea reinfection among children in Ghana.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Overview of Diarrhea Disease

Diarrhea is defined as the passage of more than 3 looser than normal stools over a 24 hour period.¹² The stools could be either watery or in addition contain some blood or mucus. The diarrhea disease could be either acute or chronic: An acute diarrhea usually has a duration of 1 or 2 days whilst, chronic diarrhea is usually greater than 14 days in duration. The symptoms of chronic diarrhea may be continual or they may be recurring. Any duration of diarrhea episode may cause dehydration, which will result in loss of essential electrolytes needed to function properly. People of all ages are susceptible to the diarrhea disease.¹⁶

An estimated 2.5 billion cases of diarrhea occur among children under five years of age each year, and estimates suggest that overall incidence comparatively remained stable over the past two decades.^{17, 18} Africa and South Asia account for more than half of these cases (Figure 1), where bouts of diarrhea are more likely to result in death or other severe outcomes. The variation of incidence of diarrhea diseases is greatly accounted for by the seasons and a child's age. The most vulnerable are youngest children: Incidence is highest in the first two years of life and as a child grows older it declines.

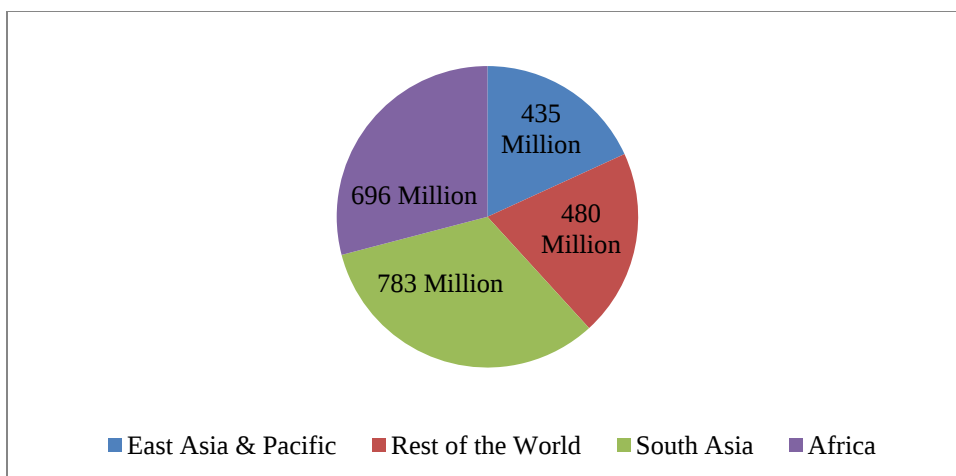


Figure 1: Proportional distribution of diarrhea cases among children under five years of age, by region, 2004 [Source: Based on World Health Organization, Global Burden of Disease estimates, 2004 update. The proportional distribution for UNICEF regions was calculated by applying the WHO cause of death estimates to the most recent estimates for the total number of under-five deaths (2007)].

Diarrhea related deaths are the second leading cause of mortality in children younger than five years in most developing countries, with an estimated 1.3 million deaths occurring worldwide annually of which 800 000 deaths occur in Africa.^{15, 19, 20}

Bacterial infections, viral infections, parasites, functional bowel disorders, intestinal diseases, food intolerances and sensitivities, and reaction to medicines are the most common causes of diarrhea.²¹ There are several types of bacteria which can infect us through contaminated food or water. The most common of these include *Campylobacter*, *Salmonella*, *Shigella*, and *Escherichia coli* (*E. coli*). There are many viruses responsible for causing diarrhea; these viruses include rotavirus, norovirus, cytomegalovirus, herpes simplex virus, and viral hepatitis. The rotavirus infection is the most common cause of acute diarrhea in children and the very elderly.²²

Rotavirus gastroenteritis is a major contributor to the overall burden of diarrhea disease in Africa. Close to 40% of hospital admissions as a result of diarrhea in children below the ages of five years are traceable to rotavirus.^{15, 23} Six of the seven countries with the highest mortality from rotavirus (>500 deaths per 100000 livebirths) are in sub-Saharan Africa, where nearly 240 000 rotavirus-related deaths out of the global yearly estimate of 527 000 occur.^{15, 24, 25}

The most common cause of acute childhood diarrhea in both developed and developing countries are rotaviruses.²⁶ In 2001, the rotavirus-induced diarrhea episodes that occurred globally was around 125 million, resulting in 500,000 to 600,000 deaths.^{27, 28}

Diarrhea may be accompanied by the following symptoms; cramp, abdominal pain, nausea, or loss of bowel control which sometimes require an urgent need to use the bathroom. However, some infections that cause diarrhea can also cause a fever and chills or bloody stools. Dehydration in children, older adults, and people with weakened immune systems is particularly dangerous. Due to this danger, dehydration must be treated promptly to avoid serious health problems, such as organ damage, shock, or coma.²¹ Rotavirus-positive episodes of diarrhea are tended to be more acute, to be associated with vomiting, caused greater dehydration, and were more likely to require hospitalization.⁵

Some common dehydration in signs infants and young children below five years include dry mouth and tongue, no tears when crying, no wet diapers for 3 hours or more, sunken eyes, cheeks, or soft spot in the skull, high fever, and listlessness or irritability. These

signs dehydration makes rotavirus diarrhea reinfections a serious problem for patients and parents/guardians alike.

In terms of diarrhea prevention, most of the efforts are concentrated on rotavirus since it is a major cause of morbidity and mortality in children. In the USA, two oral vaccines have been approved by the USA Food and Drug Administration to protect children from rotavirus infections. The two vaccines are rotavirus vaccine which is live, oral, pentavalent (RotaTeq) and rotavirus vaccine which is also live and oral (Rotarix). RotaTeq is given to infants in three doses at 2, 4, and 6 months of age. Rotarix is given in two doses. The first dose is given when infants are 6 weeks old, and the second is given at least 4 weeks later but before infants are 24 weeks old.¹⁵

Due to the established efficacy of these vaccines in the USA, European Union (EU), and other countries, the WHO intends to include RotaTeq in the EPI programmes in most African countries. Therefore, the WHO Expert Committee on Biological Standardization has recommended “new” rotavirus efficacy should be demonstrated in various geographical regions especially developing countries before a wider implementation takes place.²⁹

2.2 Rotavirus Genotypes

The human group A rotaviruses are the major etiological agents accountable for acute diarrhea in children under the age of 5 years globally. The high rates of morbidity throughout the world and mortality in developing countries, accounting for more than 500,000 infant deaths per year, at least before vaccine introduction is associated with the infection.²⁴ The classification of rotavirus strains into G-types and P-types is based on the genetic and antigenic diversity of the two outer capsid VP7 and VP4 proteins respectively.³⁰ The two proteins are independently able to elicit neutralizing antibodies and induce protective immunity. Decades ago, rotavirus molecular genotyping has demonstrated the diversity of the VP7 and VP4 strains going around the world.³¹ In recent times, there has been a proposed 11 genome segments sequence-based advanced form of the original genotyping concept.³² Among the group A rotaviruses, there are at least 23 different G genotypes (15 G serotypes) and 31 different P genotypes (15 P serotypes) have been identified.^{31 - 40} The incidence variation and distribution of rotavirus G-types and P-types between geographical areas during a rotavirus season and from one season to the next is a common feature in rotavirus epidemiology.^{5, 31} The G-types and P-types combinations, G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8], are the five most commonly associated with human infections globally.^{30,31} In addition, the existence of different genotypes is not limited to genes encoding structural proteins.^{41,46}

The two vaccines currently in use by US, EU, etc., are Rotarix1 (GlaxoSmithKline, Rixensart, Belgium), containing a single human G1P[8] rotavirus strain, and RotaTeq1 (Merck & Co., Whitehouse Station, NJ), containing 5 human-bovine reassortant rotavirus strains expressing five human serotypes (G1, G2, G3, G4, and P[8]) have

currently been licensed whiles licensing trials in other countries have been completed. These vaccines have been reported to stimulate significant protection against severe diarrhea caused by homotypic and heterotypic rotavirus strains⁴³⁻⁴⁵ and reduce childhood deaths.⁴⁶

The epidemiological surveys of the spread of rotavirus types are critical in developing countries to determine the protective efficacy of rotavirus vaccines against multiple serotypes and to detect the eventual emergence of antigenically different strains.¹⁵ A monitoring of rotavirus infection conducted in the past two decades in Venezuela showed the clinical importance of rotavirus disease in children, and rotavirus was also recognized as the major cause of death due to the diarrhea.^{47,48} A clinical trial conducted in Latin America to determine efficacy, safety and immunogenicity of the Rotarix1 vaccine in 2001–2002, showed that G1 strains predominated, but multiple rotavirus genotypes also circulate in Venezuela.⁴⁹

2.3 Reinfection of Rotavirus Diarrhea

Re-infection is common and the first infection is most severe and subsequent ones less severe.¹¹ The most common cause of severe diarrhea among children is rotavirus. Until the introduction of rotavirus vaccines in the United States in 2006, approximately 55,000 U.S. children are hospitalized each year as a result of rotavirus.⁴⁹ Globally, rotavirus is estimated to cause 527,000 deaths in children annually.⁵⁰ The incubation period for rotavirus disease is approximately 2 days. Rotavirus disease is characterized by vomiting and watery diarrhea for 3 to 8 days, and fever and frequent occurrence of abdominal pain. There is incomplete immunity after infection; however repeated infections tend to be less severe than the first rotavirus infection. Children who are vaccinated and unvaccinated may develop rotavirus disease more than once due to many different types of rotavirus and because neither vaccine nor natural infection provide full immunity (protection) from future infections.

The characteristic of a rotavirus is a wheel-like appearance when viewed by electron microscopy (the name rotavirus is derived from the Latin *rota*, meaning "wheel"). The rotaviruses are non-enveloped and double-shelled viruses.²⁹

The epidemiology of rotavirus shows that the rotavirus uses the fecal-oral route as primary mode of transmission. Since the virus is stable in the environment, transmission can occur through ingestion of contaminated water or food and contact with contaminated surfaces or objects. The USA and other countries with a temperate climate, the disease has a seasonal pattern, with annual epidemics occurring from December through June. The highest rates of rotavirus illness occur among infants and young children.

The diagnosis of rotaviruses may be made by rapid antigen detection of rotavirus in stool specimens. The strains of the rotavirus may be further characterized by enzyme immunoassay or reverse transcriptase polymerase chain reaction, but this testing is not commonly performed.

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study Location

The original study was conducted in the Kassena – Nankana District (KND) in northern Ghana. The KND lies within the Guinea Savannah woodland of northern Ghana and are among the districts with the worst social economic status in the country. The district covers a land area of 1675 square kilometers and shares borders with Burkina Faso in the Upper East region of Ghana. The KND has a population of 140000 people living in approximately 13000 dispersed compounds. The KND is one of the most arid districts in northern Ghana with a long dry season punctuated with only three months of rains and average temperatures ranging between 20 and 40 degrees Celsius. Lack of nutritious meal is common in the KND worsening the morbidity and mortality of infectious diseases. Poverty and economic challenges has been retarding efforts to improve health conditions in the district. There is the existence of evidence that the Upper East region of Ghana is among the poorest regions in Ghana, as a result, the government of Ghana has designated the KND as a special research zone for assessing health problems in the area and practical means of assessing them. The KND has low levels of education and literacy.⁵⁹

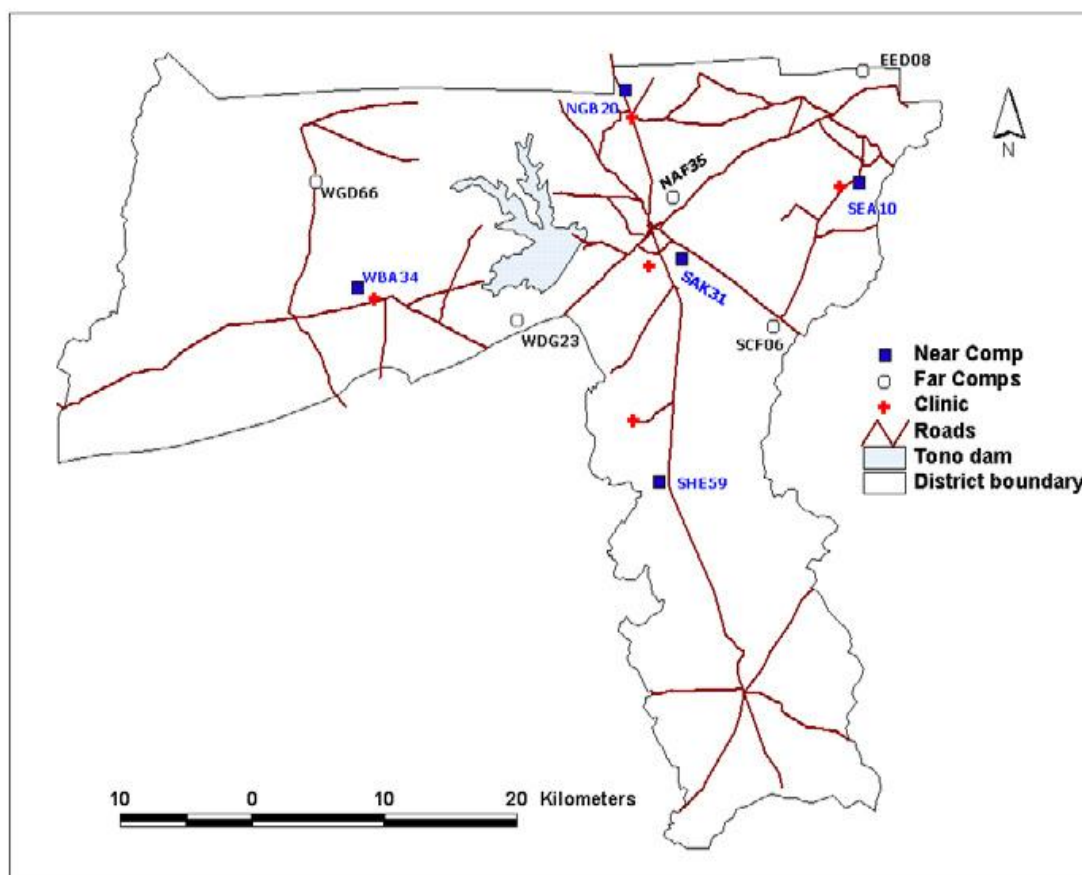


Figure 2: Map of Kassena – Nankana District (KND) Showing Location of Health Facilities [Source: Owusu-Agyei et al. (2007). Assessing malaria control in the Kassena – Nankana district of northern Ghana through repeated surveys using the RBM tools]

3.2 Study Design

The study was a randomized, double-blinded, placebo-controlled trial to be conducted in Ghana to estimate rotavirus diarrhea reinfection and key factors associated with the reinfection among children in Ghana.

3.3 Sample Size and Power

The number of rotavirus diarrhea reinfections was estimated with its associated 95% confidence interval. The type I error (α) was controlled at $\alpha = 0.025$ (one-sided). Based on the assumption that the true reinfection was 75% and the captured attack rate was 1.5% with an unevaluable rate of 20% of subjects, a sample size of 5468 (1RotaTeq: 1 placebo) provided approximately 93% power to declare rotavirus diarrhea reinfection significant. This assumption was based on the Efficacy, Safety, and Immunogenicity of RotaTeq among Infants in Asia and Africa study in Ghana.²⁹

3.4 Research Questions

1. How many children have had rotavirus diarrhea reinfection after the first dose of vaccination?
2. What is the predominant rotavirus genotype that causes diarrhea reinfection among children in Ghana?
3. What is the effect of the number of severe rotavirus diarrhea reinfection on the weight of child at study entry?
4. What is the effect of the number of repeated severe rotavirus diarrhea episodes on hospitalization – duration?
5. Could the following confounding variables:
 - i. Rotavirus genotype response diarrhea reinfection
 - ii. Child's age at first dose of vaccination

- iii. Sex of child and
- iv. Trial Arm of child has an association with the effect of severe rotavirus diarrhea reinfection on its outcomes?

3.5 Data Source and Variables

The data set that was used for this analysis contained information on 2227 children who participated in the Efficacy, Safety, and Immunogenicity of RotaTeq among Infants in Asia and Ghana study in Ghana.

The data set was collected from the principal investigator in Ghana for this study. The whole data set was available for maximum statistical output since the sample size was large enough to do an epidemiological analysis. The following variables were analyzed in the study:

3.5.1 Outcome Variables

- i. Weight of child at study entry and
- ii. Hospitalization – Duration

3.5.2 Exposure Variable

- i. Number of Rotavirus Diarrhea Reinfection after first dose of vaccination

3.5.3 Confounding Variables

- i. Rotavirus genotype response diarrhea reinfection
- ii. Child's age at first dose of vaccination
- iii. Sex of child
- iv. Trial Arm of child.

3.6 Data Collection Methods

A Case Report Forms (CRFs) were used in the primary data collection. The study was based at the designated study health facilities. Trained field staff assigned to the designated health facilities screened the children and mothers/guardians to make sure that the inclusion and exclusion criteria of the study are met.

For this study the inclusion criteria was children with one or more gastroenteritis (particular focus on diarrhea) after first dose of vaccination.²⁹

3.7 Laboratory Methods

3.7.1 Rotavirus Antigen Detection Assay

The purpose of the Rotavirus Antigen Detection Assay was to detect rotavirus in stool before and after vaccination with the rotavirus content vaccine. The results for the assay were reported as positive (+) or negative (-) for the presence of rotavirus.

The assay is valid if a Plate Control is found to be positive by both cut – off rules for positive and negative samples (positive ODs must be $>.31$ and must be at least 1.63 fold than negative ODs) and the maximum/minimum ratio of the duplicate OD readings for the control should not exceed 1.67 (or 1.23).²⁹

3.7.2 Rotavirus VP6 Assay

The purpose of the rotavirus VP6 assay was to determine the VP6 genotype of any rotavirus present in stool samples. Samples from patients with acute gastroenteritis were first evaluated for the presence of rotavirus antigen in the stool samples and all antigen – positive samples were then evaluated to distinguish between wild type rotavirus strains from vaccine – virus strains. The results for the assay were reported as the species with the closest identity to the resulting sequence.²⁹

3.7.3 Rotavirus VP7 Serotyping Assay

The purpose of the rotavirus VP7 serotyping assay was to determine the VP7 (G) genotype of any rotavirus present in stool samples. The rotavirus VP7 serotyping assay was used in the original study to determine the rotavirus VP7 genotypes (G – types) in biological samples. The results for the assay are reported as G genotypes, for example, G1, G2, G3, G4, G5, G8, G9, G10, or G12.²⁹

3.7.4 Rotavirus VP4 Serotyping Assay

The purpose of the rotavirus VP4 serotyping assay was to determine the VP4 (P) genotype of any rotavirus present in stool samples. The rotavirus VP4 serotyping assay was used to determine the rotavirus VP4 genotypes (P – types) in biological samples. The results for the assay were reported as G genotypes, for example P1A [8], P1B [4], P2A [6], P2B [6], P7 [5], etc. Precision for both VP7 and VP4 was defined as a measure of the assay's ability to reproduce a test sample result across independent runs of the procedure (inter – assay) and between analysts. The assay validity criteria was based on the assay controls and evaluated at several steps in the assay.²⁹

3.8 Outcome Measures

The secondary analysis of the dataset focused on the estimation and determination of the effect of the number of rotavirus diarrhea reinfection on weight of child at study entry and hospitalization – duration among the children after the first dose of vaccination. The reinfection among the children was estimated as a child having diarrhea episode after first dose of vaccination.

The confounding factors associated with severe rotavirus diarrhea reinfection were considered in the subgroup analysis.

3.9 Statistical Analysis Plan

3.9.1 Data Description and Extraction

The baseline data set was used to explore the children's baseline characteristics and variations using simple description methods of analysis such as summary statistics and frequency distributions.

All the variables were identified and described. Data quality checks were performed to screen for any outliers and missing values.

3.9.2 Categorization of Variables

Continuous variables such as age were categorized into three (3) groups for interpretation of results; age 4-6 weeks, age 7-9 weeks and age 10-12 weeks. The number of rotavirus diarrhea reinfection was categorized into one reinfection, two reinfections, and three reinfections. Sex and trial arm of the child both had a binary outcome; sex being male or female and trial arm being treatment or placebo arm. The rotavirus genotype responsible for the diarrhea reinfection was grouped according to the G genotypes.⁵⁵

Children were considered to have reinfection of rotavirus diarrhea if they had any episode of diarrhea after first dose of vaccination. Two (2) outcomes (weight of child at study entry and hospitalization – duration) were the only outcome variables that were determined.

3.9.3 Missing Data Management

Children with missing baseline data were excluded from the analysis.

3.9.4 Statistical Analysis

The baseline data that was analyzed included age (weeks), sex, weight, and height. Severe rotavirus diarrhea reinfection was described as any diarrhea episode after first dose of vaccination against rotavirus diarrhea.

3.9.5 Definition for Measure of Association

Linear regression was used to measure the effect of number of repeated severe rotavirus diarrhea episodes on weight of child at study entry whiles confounding for rotavirus genotype responsible for diarrhea reinfection, child's age at first dose of vaccination, sex of child, and trial Arm of child.

Logistic regression was also used to measure the effect of the number of rotavirus diarrhea reinfection on hospitalization – duration whiles confounding for rotavirus genotype responsible for diarrhea reinfection, child's age at first dose of vaccination, sex of child, and trial Arm of child.

The Logistic regression is the appropriate statistical measure of the effect because it allows more flexibility in examining the effects of the exposure variable which is categorical.

3.9.6 Methods for Analysis

The statistical software package Stata version 10 was used throughout the data cleaning and the analysis.

3.9.7 Identifying Risk Factors

A univariate linear and logistic regression model was fitted to measure the effect each of the exposure variables on either outcome variables. The exposure variable or any confounder having P-value ≤ 0.05 was included in the regression model.

3.10 Analysis of Data

The Stata version 10 software was used during the data analysis. Simple proportions and confidence intervals were used to estimate rotavirus diarrhea reinfection rate among children in Ghana. Again, simple proportions were used to determine the dominant rotavirus genotype that causes diarrhea reinfection among children in northern Ghana using the genotype data.

In order to determine the confounding variables (rotavirus genotype responsible for diarrhea reinfection, child's age at first dose of vaccination, sex of child, and trial arm of child) associated with rotavirus diarrhea reinfection among children in Ghana, the Linear and Logistic Univariate Regression Analysis was performed to test for an association between each factor and average weight change or hospitalization – duration among the children in northern Ghana.

3.11 Limitations

Apart from normal limitations of secondary data used to answer more research questions other than its primary and secondary endpoints. The data answered all the research questions required in this study except for feeding status of the children that was not available as a risk factor average weight change.

3.12 Ethical Considerations

The Ghana Health Service Ethical Approval: Ethical clearance was obtained from the Ghana Health Service Ethical Review Committee of the Research and Development Division of the Ghana Health Service (GHS-ERC: 62/03/12).

Approval from Principal Investigator: Verbal approval was sought from the Principal Investigator of the Efficacy, Safety, and Immunogenicity of RotaTeq among Infants in Asia and Africa study in Ghana for the use of the data for a secondary analysis in this study.

Description of Subjects Involved in The Study: There wasn't any vulnerable participant involved in this study, nor were there any risk to anyone in the earlier study. Information provided on participants involved in the secondary analysis was kept confidential.

Privacy and Confidentiality: was exercised in handling the participant's information provided.

Voluntary Consent: Participation in the study was voluntary and the participants were free to refuse to answer any question(s) and could opt out of the study at any time of the study.

Conflict of Interest: I hereby declare that there was no conflict of interest apart from the academic and public health relevance of this study. I also declare that this study was self – sponsored and an amount of about US\$1035.00 was spent in carrying out this study.

Contact Information: If there are any question(s) about this study, please direct them to Mr. Clement T. Narh (Principal Investigator) School of Public Health, College of Health Sciences University of Ghana, Legon.

Mobile: 0265540346/0243104190

Email: iteetee@gmail.com

Or

Academic Supervisor: Prof. George E Armah

Lecturer, School of Public Health (SPH)

Noguchi Memorial Institute for Medical Research (NMIMR)

Tel.: 0208246513

Email: garmah@noguchi.mimcom.org

Contact of Ethics Review Committee Administrator

Ghana Health Service Research Ethics Committee

Ghana Health Service

Post Office GP 184

Accra, Ghana

Phone: 233 302 681109

Fax: 233 302 226739

Email: Hannah.Frimpong@ghsmail.org

CHAPTER FOUR

4.0 RESULTS

4.1 Introduction

The results chapter presents the baseline characteristics of the trial participants and the strains of rotavirus genotypes, unadjusted linear and logistic regressions, adjusted linear and logistic regressions and a box plot.

4.2 Baseline Characteristics of Study Participants

The RotaTeq Ghana study dataset contained information on 2227 children of which 27 were excluded because they were older than 12 weeks of age. 1098 children were randomly assigned to the Vaccine arm and 1102 children to the Placebo arm.

Of the three age groups of children in this study, almost 45% of the children in both arms were in the age group 7 – 9 weeks. The proportion of females to males in each arm was very similar.

Table 1: Baseline Characteristics of Study Participants

Characteristics	Vaccine Arm Number (%)	Placebo Arm Number (%)
Number of Infants Randomly Assigned	1098 (49.9%)	1102 (50.1%)
Age at Randomization (weeks)		
4-6	297 (27.1%)	314 (28.5%)
7-9	511 (46.5%)	493 (44.7%)
10-12	290 (26.4%)	295 (26.8%)
All	1098 (100%)	1102 (100%)
Sex		
Female	556 (50.6%)	557 (50.5%)
Male	542 (49.4%)	545 (49.5%)
All	1098 (100%)	1102 (100%)
Mean Weight at Enrollment	4.6 (1.0) *	4.6 (0.9)*
Mean Height at Enrollment	58.2 (3.6)*	57.9 (3.4)*
* Data are mean (SD) unless otherwise stated		

Other baseline characteristics such as weight of the children at randomization, and height at trial entry were similar between the vaccine and placebo arms of the study as shown in Table 1.

4.3 Distribution of Rotavirus Severe Diarrhea Episodes, Detected Genotypes and Hospitalization

There were a cumulative total of 142 diarrhea infections in the Vaccine arm and 151 in the Placebo arm. Children in the age group 7 – 9 weeks suffered weight loss and hospitalization during the first severe diarrhea episode (66/142 in the vaccine arm and 69/151 in the placebo arm). The same age group of children was mostly hospitalized as shown in Table 2. During the study, the maximum number of times a child had repeated episodes of diarrhea was three times in the Vaccine and two times in the Placebo arms respectively. Fifty-four percent (54%) of all children who had diarrhea were hospitalized and 46.0% were outpatients in the vaccine arm while inpatients were 59.7% and outpatients were 40.3% in the Placebo arm respectively.

The genotypes of rotaviruses identified in diarrhea stools in the Vaccine arm were G1 (40.1%), G2 (46.5%), G3 (11.3%), and G8 (2.1%), whilst in the Placebo arm the genotypes identified were G1 (31.8%), G2 (45.0%), G3 (13.2%), and G8 (9.9%). There was no statistical evidence to show that the rotavirus genotype that causes that first diarrhea infection is different from the subsequent infections in Figure 2.

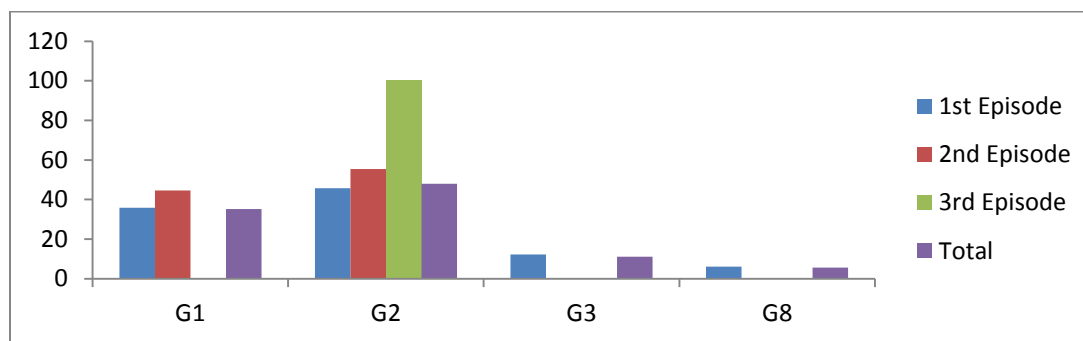


Figure 3: Rotavirus Genotypes and Number of Diarrhea Episodes

Table 2: Distribution of Rotavirus Severe Diarrhea Episodes, Detected Genotypes and Hospitalization

Characteristics	Vaccine Arm			Placebo Arm		
	Age group (Weeks)			Age group (Weeks)		
	4 – 6	7 – 9	10 – 12	4 – 6	7 – 9	10 – 12
Number of Severe Diarrhea Episodes	Number (%)			Number (%)		
0	253 (85.2%)	434 (84.9%)	253 (87.2%)	262 (83.4%)	418 (84.8%)	257 (87.1%)
1	42 (14.2%)	66 (12.9%)	34 (11.7%)	46 (14.7%)	69 (14.0%)	36 (12.2%)
2	1 (0.3%)	9 (1.8%)	3 (1.0%)	6 (1.9%)	5 (1.0%)	2 (0.7%)
3	1 (0.3%)	2 (0.4)	-	-	1 (0.2)	-
All	297 (100%)	511 (100%)	290 (100%)	314 (100%)	493 (100%)	295 (100%)
Hospitalization						
Inpatient	6 (2.0%)	6 (1.2%)	4 (1.4%)	5 (1.6%)	10 (2.0%)	4 (1.4%)
Outpatient	291 (98.0%)	505 (98.8%)	286 (98.6)	309 (98.4%)	483 (98.0%)	291 (98.6%)
All	297 (100%)	511 (100%)	290 (100%)	314 (100%)	493 (100%)	295 (100%)
Detected Genotypes						
G1	57 (40.1%)			48 (31.8%)		
G2	66 (46.5%)			68 (45.0%)		
G3	16 (11.3%)			20 (13.2%)		
G8	3 (2.1%)			15 (9.9%)		
All	142 (100%)			151 (100%)		

4.4 Unadjusted Linear Regression for Average Weight Change of Study Participants

Table 3 shows the univariate (unadjusted) linear regression of the average weight change (outcome), number of rotavirus infections and other baseline characteristics for the Vaccine and Placebo arms respectively. Of all the baseline variables included in the univariate analysis, there is strong evidence (P-value <0.001) of an associated reduction in the average weight for multiple rotavirus infections in both the vaccine and placebo arm of the trial. Children randomized at between 7 – 9 weeks and age group 10 – 12 weeks of age were observed to have a greater reduction in weight than the other age groups (P-Value=0.012 and P-value <0.001 respectively). However, in the placebo there was no statistical evidence of reduced average weight in the age group 10 – 12 weeks group. There was no association between the sex of the children and the infecting genotypes as shown in Table 3.

Table 3: Unadjusted Linear Regression of Study Participants

Average Weight	Vaccine Arm		Placebo Arm	
	Regression Coefficient (95% CI)	P-Value	Regression Coefficient (95% CI)	P-Value
Number of Severe Diarrhea Episodes				
1	-2.42 (-2.70, -2.15)	<0.001	-2.33 (-2.59, -2.07)	<0.001
2	-3.47 (-4.31, -2.64)	<0.001	-3.28 (-4.13, -2.64)	<0.001
3	-3.39 (-5.12, -1.66)	<0.001	-3.45 (-6.37, -0.52)	0.021
Constant	4.80 (4.69, 4.91)	<0.001	4.75 (4.64, 4.86)	<0.001
Age at Randomization (weeks)				
7-9	-0.37 (-0.67, -0.08)	0.012	-0.4 (-0.32, 0.24)	0.80
10-12	-0.54 (-0.86, -0.22)	<0.001	-0.47 (-0.78, -0.16)	<0.001
Constant	4.66 (4.43, 4.89)	<0.001	4.44 (4.22, 4.66)	<0.001
Sex				
Male	0.07 (-0.17, 0.31)	0.58	0.07 (-0.16, 0.31)	0.54
Constant	4.31 (4.14, 4.48)	<0.001	4.26 (4.09, 4.43)	0.001
Detected Genotypes				
G2	-0.56 (-1.83, 0.71)	0.38	-0.42 (-1.29, 0.45)	0.34
G3	0.28 (-1.89, 2.44)	0.80	-0.68 (-1.92, 0.56)	0.28
G8	0.11 (-3.91, 4.12)	0.96	-0.15 (-1.50, 1.19)	0.82
Constant	4.39 (3.45, 5.34)	<0.001	4.09 (3.43, 5.43)	<0.001

4.4 Unadjusted Logistic Regression for Hospitalization of Study Participants

There is strong evidence of an association between number of diarrhea infection and the length of hospitalization among the children in both the Vaccine and Placebo arms (LR: P-value <0.001). The odds (OR=11.16) of having at least one diarrheal infection is similar among children in both the Vaccine and Placebo arms is 11 times that of those who were not admitted with diarrhea infection. Similarly, the odds of having two infections is about 15 times the odds of hospitalization of children without diarrhea when Vaccine was administered as compared to the 28 times odds of hospitalization of children in the Placebo arm. It appears that the odds of the length of hospitalization increases with the number of infections. With regards to age, there is no statistical evidence of association between ages and hospitalization in both arms since none of the 95% Confidence Intervals (95% CIs) contains the null value (1 of independence). Sex of the children in the Vaccine arm shows statistical evidence (P-value=0.03) of an association with hospitalization but not in the Placebo arm (P-value=0.23). The odds of hospitalization – duration among males is about 3 times that among females in the Vaccine arm. The infecting rotavirus genotypes have no association with hospitalization – duration irrespective of the trial arm in Table 4.

Table 4: Unadjusted Logistics Regression for Hospitalization – Duration of Study Participants

Hospitalization– Duration	Vaccine Arm		Placebo Arm	
	Odds Ratio (95% CI)	LR: P-Value	Odds Ratio (95% CI)	LR: P-Value
Number of Severe Diarrhea Episodes				
1	11.16 (3.60, 34.63)	<0.001	11.00 (3.94, 30.75)	<0.001
2	15.58 (1.69, 143.66)	<0.001	28.21 (5.12, 155.52)	<0.001
3	374 (29.02, 4820.05)	<0.001	-	-
Age at Randomization (weeks)				
7-9	0.58 (0.18, 1.80)	0.64	1.28 (0.43, 3.78)	0.76
10-12	0.68 (0.19, 2.43)	0.64	0.85 (0.23, 3.19)	0.76
Sex				
Male	3.12 (1.00, 9.75)	0.03	1.77 (0.69, 4.53)	0.23
Detected Genotypes				
G2	0.33 (0.07, 1.60)	0.36	1.89 (0.34, 10.58)	0.80
G3	0.50 (0.05, 5.51)	0.36	2.78 (0.34, 22.75)	0.80
G8	-	-	1.79 (0.14, 22.70)	0.80

4.6 Adjusted Linear and Logistics Regression for Age and Sex of Study Participants

After adjusting for age group in a bivariate linear regression analysis in Table 5, number of infection shows statistical evidence (P-value <0.001) of having an association with average weight change and age group 7 – 9 weeks and age group 10 – 12 weeks also showed similar statistical evidence of an association in the Vaccine arm and in the Placebo arm, age group 7 – 9 weeks did not again show any statistical evidence of a reduction in the average weight change, however, the regression coefficient for age group 7 – 9 weeks changed from -0.4 (95% CI=-0.32, 0.24) to -0.08 (95% CI=-0.31, 0.15) in the Placebo arm.

In the adjusted logistic regression, sex of the children in both the Vaccine and Placebo arms of the study were not having any statistical evidence of an association with hospital – duration of the children who had rotavirus diarrhea after first dose of vaccination (P-value=0.068 and P-value=0.305) respectively.

Table 5: Adjusted Linear and Logistics Regression for Age and Sex of Study Participants

Linear Regression				
Average Weight	Vaccine Arm		Placebo Arm	
	Regression Coefficient (95% CI)	P-Value	Regression Coefficient (95% CI)	P-Value
Number of Severe Diarrhea Episodes				
1	-2.44 (-2.72, -2.17)	<0.001	-2.35 (-2.61, -2.09)	<0.001
2	-3.43 (-4.25, -2.60)	<0.001	-3.36 (-4.20, -2.64)	<0.001
3	-3.49 (-5.20, -1.78)	<0.001	-3.57 (-6.46, -0.68)	0.016
7-9 (Age in weeks)	-0.33 (-0.57, -0.09)	0.007	-0.08 (-0.31, 0.15)	0.500
10-12 (Age in weeks)	-0.62 (-0.89, -0.35)	<0.001	-0.59 (-0.85, -0.32)	<0.001
Constant	5.12 (4.92, 5.32)	<0.001	4.95 (4.76, 5.14)	<0.001
Logistic Regression				
Hospitalization–Duration	Odds Ratio (95% CI)	P-Value	Odds Ratio (95% CI)	P-Value
Number of infection				
1	11.03 (3.54, 34.33)	<0.001	11.41 (4.07, 32.00)	<0.001
2	15.10 (1.61, 141.55)	0.017	24.95 (4.46, 139.71)	<0.001
3	370.12 (26.47, 5174.43)	<0.001	-	-
Male	3.03 (0.92, 10.01)	0.068	1.68 (0.62, 4.50)	<0.305

The Box plot in Figure 1 shows the weight of the children at the trial entry (weight0), weight at first infection (weight1), weight at second infection (weight2), weight at third infection (weight3), and the average weight change of all the weights at each infection (average weight change). The Box plot also shows the range and distribution of the weights.

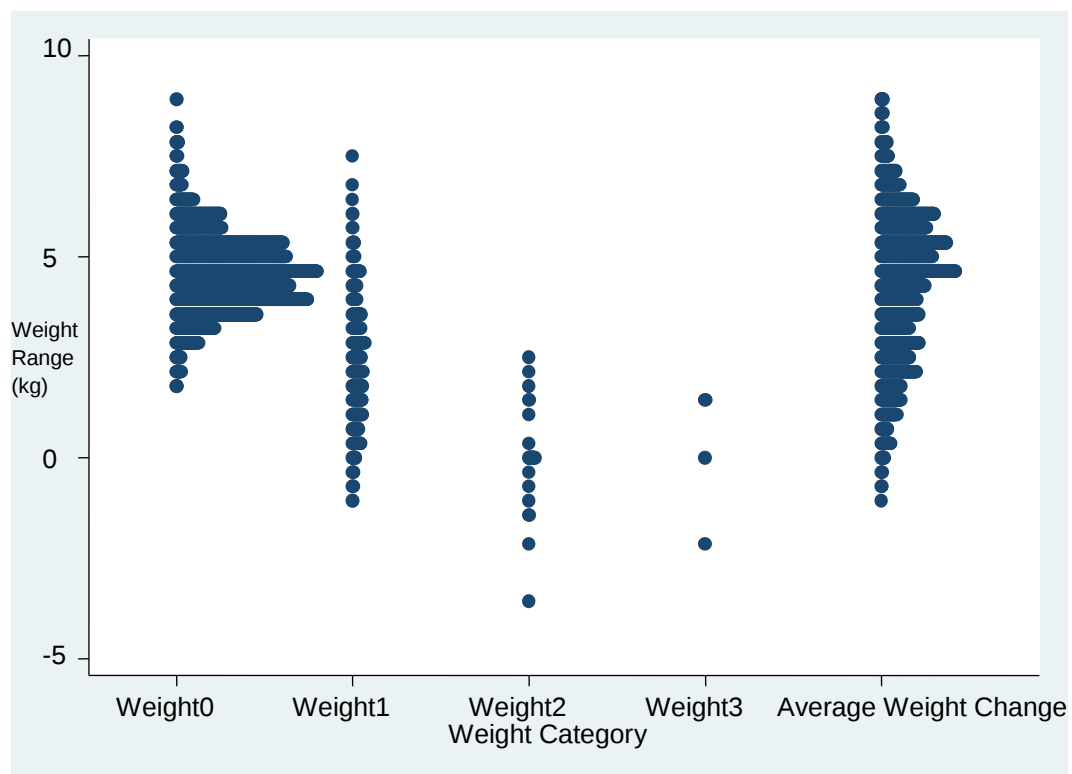


Figure 4: Dot Plot of the Effect of Number of Rotavirus Diarrhea Reinfection on Weight of Ghanaian Children in the Rotavirus Diarrhea Reinfection and Its Outcome on Weight and Hospitalization – Duration at Study Entry

CHAPTER FIVE

5.0 DISCUSSION

5.1 Introduction

The study was a randomized, double-blinded, placebo-controlled trial to investigate severe rotavirus diarrhea reinfection and its outcome on weight and hospitalization – duration among 2200 Ghanaian children during the first 106 weeks of life - duration among children in the Kassena Nankana District of Ghana. The discussion will cover the following section rotavirus diarrhea reinfection among children in northern Ghana, rotavirus genotypes that causes severe diarrhea reinfection requiring hospitalizations among children in northern Ghana and Comparism of outcomes (average weight change of child in the study and Hospitalization – Duration) in each trial arm.

5.2 Rotavirus Severe Diarrhea Episode and Reinfection

Of the 1098 children in the Vaccine arm of the trial, about eighty – five percent of the children were protected from any severe diarrhea episode during their first two years of life. This protection was similar (85%) among the children in the Placebo arm as well. Out of the remaining fifteen percent of the children who had between one and three episodes of severe diarrhea, thirteen percent had one episode in the Vaccine and fourteen percent in the Placebo. This means that the first episode of severe diarrhea was more in the children who were vaccinated with the placebo. The distribution of two episodes of

severe diarrhea were similar among the children in both the Vaccine and Placebo arms with the third infection in the Vaccine arm being three times that of the Placebo.

Hospitalization – duration of the children who had any number of diarrhea infections was categorized into a binary outcome as an Inpatient or Outpatient. Although, in both arms the most of the children who an infection were treated as inpatients; More children in the Placebo arm (56%) require hospitalization for their infections compared to that of the Vaccine arm (54%).

5.3 Rotavirus G Genotypes

The rotavirus G genotypes that were present in the stools of children who had diarrhea infection were G1 (40.1%), G2 (46.5%), G3 (11.3%), and G8 (2.1%) in the Vaccine arm and G1 (31.8%), G2 (45.0%), G3 (13.2%), and G8 (9.9%) in the Placebo arm. In both arms of the trial the G2 genotypes of the rotavirus caused more infections, followed by the G1 genotype. In terms of hospitalization – duration and the strains of genotypes in the G2 of the rotavirus dominated the others in both arms (Vaccine – 51.4% and Placebo 44.6%) for outpatients. In the Vaccine arm most of the hospitalized children were as a result of the G1 strain (60.0%), whiles G2 (50.0%) again was responsible for hospitalization in the Placebo arm. The study showed no evidence of different G genotypes causing any reinfection.

5.4 Comparism of outcomes (average weight change of child in the study and Hospitalization – Duration)

The predominance of weight loss for the first diarrhea infection is consist with most studies on diarrhea dehydration especially Ruuska and Vesikari in 1991⁵⁸ and Velazquez et al in 1996¹¹. There is 2.44 average weight loss in the children at their first infection compared to about 3.49 weight loss on the second and third infections in both arms. The children in the age group 7 – 9 weeks old lost 0.33 (P-value=0.007) weight compared to the children in the age group 4 – 6 weeks which is a significant weight reduction in the Vaccine arm and in the Placebo arm the children in the age group 7 – 9 weeks old lost 0.08 (P-value=0.50) weight compared to the children in the age group 4 – 6 weeks which was not significant. About 0.62 (P-value<0.001) reduction in the weight of the children was recorded in the age group 10 – 12 weeks compared to the age group 4 – 6 weeks in both Vaccine and Placebo arms which are significant reductions in weight after adjusting for age. It was also clear in the Box plot that much weight was lost on the second and third infection compared those who had one infection.

The strong evidence (P-value <0.001) of an association that any number of diarrhea have with hospitalization – duration of the children means that irrespective of the number of infections that a child gets they will be a hospitalized. This hospitalization – duration increases with an increase in the number of diarrhea infections. Though, the number of infection increases hospital – duration, the hospital – duration for a child vaccinated with Placebo (OR=24.95) is almost twice that of the child vaccinated with RotaTeq vaccine (OR=15.10) after adjusting for the age of these children which did not show any

statistical evidence of increasing hospitalization – duration in the bivariate analysis in either arm. These findings of hospitalization as a result of diarrhea episodes were earlier reported by Binka et al 2003⁵ and WHO 2008²³.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

This study was a randomized, double-blinded, placebo-controlled trial investigating the outcome of rotavirus diarrhea reinfection in terms of average weight change and hospitalization–duration of northern Ghanaian children during the first 106 weeks of life. The findings of this study would help in the management of rotavirus diarrhea dehydration and hospitalization of patients.

This study has shown that the risk of getting diarrhea infection is proportional to the age of the children, therefore if children are vaccinated at an early age, the lesser the risks of an infection. There was no link for a different genotype responsible for rotavirus diarrhea reinfections; hence the prevention of the first infection would serve as prevention for subsequent infections. The study also showed that there is progression in hospitalization with increase in the number rotavirus severe diarrhea episodes.

These results have shown significant evidence of weight loss and an increase hospitalization at any severe rotavirus diarrhea infection. Using this new knowledge on the Vaccine, we are now aware with our own data on weight loss and increase in hospital admissions as a result of severe rotavirus diarrhea incidents.

6.2 Recommendation

This study makes the following recommendations:

1. Children should be provided with the Vaccine early in life since severe rotavirus diarrhea infection and hospitalization – duration varies with the age and the number of severe diarrhea episodes which is prevented in children vaccinated at early age.
2. This study suggests further studies designed with enough sample of repeated episodes of diarrhea. Since this study had fewer cases of repeated episodes of diarrhea, which is not powered enough.

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