

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

**BIRTH WEIGHT AND MORBIDITY AMONGST INFANTS WITH
ROTAVIRUS GASTROENTERITIS IN A ROTAVIRUS VACCINE TRIAL
IN KASSENA-NANKENA DISTRICT.**

BY

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DECLARATION

I, Kofi Marfo Osei 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.

Signed:


.....
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Supervisor:

.....

Professor George Armah

DEDICATION

I will like to dedicate this thesis first to the Almighty God, then to my Mother - Rebecca Osei Dankwah and then to my dear wife - Mary Adwoa Sarpong.



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I would like to acknowledge the following people who contributed in the following ways to a successful completion of this thesis.

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ABBREVIATIONS

RVGE -----	ROTAVIRUS GASTROENTERITIS
CI -----	CONFIDENCE INTERVAL
AGE -----	ACUTE GASTROENTERITIS
RCT -----	RANDOMIZED CONTROLLED TRIAL
NBW -----	NORMAL BIRTH WEIGHT
LBW -----	LOW BIRTH WEIGHT
HR -----	HAZARD RATIO
RotaTeq -----	NEW ROTAVIRUS VACCINE TESTED
WHO -----	WORLD HEALTH ORGANIZATION
IUGR -----	INTRAUTERINE GROWTH RESTRICTION
IRR -----	INCIDENCE RATE RATIO
KND -----	KASSENSA NANKANA DISTRICT
NK -----	NATURAL KILLER CELLS
IgG -----	IMMUNOGLOBULIN G
IgA -----	IMMUNOGLOBULIN A
NDSS -----	NAVRON DEMOGRAPHIC SURVEILLANCE SYSTEM
ICH -----	INTERNATIONAL CONFERENCE ON HARMONIZATION
HIV -----	HUMAN IMMUNODEFICIENCY VIRUS
SGA -----	SMALL FOR GESTATIONAL AGE
RT-PCR -----	RotaTeq POLYMERASE CHAIN REACTION
IQR -----	INTERQUARTILE RANGE

ABSTRACT

Background: Rotavirus gastroenteritis in children remains a global public health problem. It is responsible for over 2 million hospitalizations in children less than 5 years. Low birth weight is a known risk factor for several childhood infections and diseases. Over 96% of all low birth weight deliveries occur in Sub-Saharan Africa. However, the relationship between rotavirus gastroenteritis and its burden in terms of morbidity or mortality is unknown.

Objective: to investigate the relationship between the morbidity of rotavirus gastroenteritis and low birth weight.

Methodology: The primary data from the RotaTeq Trial in the Kassena-Nankana District was used to answer the research question. Some extracted variables relevant to the objective, include: age, birth weight, gender, date of randomization, time to first AGE, Rota virus antigen status and time of exit. Extracted data was scrutinized to ensure integrity and usability. Statistical methods such as proportions and percentages were used for categorical data, mean, standard deviations for continuous data and primary end point was analyzed using Kaplan Meier methods and cox regression. Stata 12 software package was used for the analysis.

Results: Data relating to 834 infants recruited were analyzed, normal birth weight (NBW) 697 and low birth weight (LBW) 137. At baseline the group was balanced for age, gender, immunization status and total duration of follow-up. 6.7% of NBW versus 6.6% of LBWs developed rotavirus gastroenteritis (RVGE), hazard ratio (HR) 0.99(0.49-2.04), p (0.99).

Subgroup analyses among unvaccinated and vaccinated group showed p 0.96 and 0.66 respectively. The incidence rate ratio (IRR) of RVGE in LBWs compared to NBWs was 0.97 (0.48-1.99), p (0.94). The Mean Vesikari score - NBW 8.30 ± 2.98 , LBW 9.22 ± 2.95 p (0.40). After adjusting for vaccination status the mean Vesikari scores, p values were not significant.

Conclusion: Birthweight may not be a risk factor for the development of RVGE within the first 2 years of life in the population studied from the rotavirus efficacy trial in Kassena-Nankana District in Navrongo.

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CHAPTER ONE

1.0 - BACKGROUND

The impact of Rotavirus Gastroenteritis (RVGE) in children over recent decades has made it a global public health problem. The prevention and control of RVGE infection depends on the success of strategies to prevent new infections and treat the currently infected. The efforts to reduce the impact of RVGE so far has been two fold:

- i. The introduction of rotavirus vaccines such as RotaTeq - randomized control trial (RCT) was conducted in Navrongo.
- ii. Prompt supportive treatment for fever and fluid resuscitation.

The introduction of vaccines over the past decade has been a panacea for prevention and reduction of morbidity and mortality associated to RVGE in children. Local data from clinical trials are beginning to demonstrate the benefits over risks.

Despite knowing the efficacy of rotavirus vaccine, predictors for severity of RVGE are not well elucidated. Like most infancy infectious diseases like malaria, birthweight has been a known predictor. It is therefore not surprising that public health efforts are directed at preventing LBW. The relationship between birth weight and severity of RVGE is nebulous. Little can be found literature concerning this likely risk factor for severity of RVGE [1, 12].

Between 2007 and 2009 a clinical trial of the RotaTeq vaccine was conducted in Navrongo in the Kassena Nankana District (KND). The objective of the trial was to determine the efficacy and

safety of the vaccine [1]. Whilst the study measured the efficacy and safety of RotaTeq in infants, it did not address the question of the relationship between birth weight and severity of rotavirus infection in children. In this study secondary data was used from the RotaTeq clinical trial in Navrongo to establish or otherwise any existing relationship between birth weight and severity of rotavirus infection.

1.1 - PROBLEM STATEMENT

Each year, rotavirus causes about 2 million hospitalizations and about 440,000 deaths in children less than 5 years old [2]. 1 out of 5 infected infants with RVGE will visit the clinic because of rotavirus gastroenteritis, 1 out of 65 will be hospitalized and 1 out of 293 will die [2].

82% of RVGE mortality occurs in developing countries. It is estimated that in 2008 alone about 453,000 children less than 5 year old died from RVGE. [3]. RVGE account for 6% of diarrheal episodes, and 20% of all diarrheal related deaths [2].

Birth weight is an important determinant of infant morbidity and mortality [4]. Favorable birth weight ranges from 3500 to 4500 g [4]. After literature search, data on the effect of birth weight on morbidity caused by RVGE in the first 2 years of life is scarce.

More than 90% of low birth weight occurs in developing countries [5], coincidentally 82% of worldwide diarrhea prevalence is in developing countries including Ghana [6].

Most childhood infections occur during the early stages of life and birth weight is a known risk factor [4]. Neonatal mortality from effects of LBWs contributes about 38% to under 5-mortality are in Ghana [7].

1.2 - RATIONALE

RVGE is recognized as a major contributor to neonatal and infant mortality. In Sub-Saharan Africa including Ghana, mortality figures from RVGE have remained high comparatively from 2003 [2], 2006 [6] and 2008 [3]. Mortality from the consequences of LBWs contributes significantly to the neonatal mortality rates and therefore the high under 5-mortality rate in sub-Saharan Africa. Ghana's under 5-mortality rate as of 2010 was 74.40 per 1000 live births and neonatal mortality rate as of 2010 was 28.00 per 1000 live births [7]. Thus neonatal mortality contributes about 38% to under 5-mortality rate, although the neonatal period constitutes only about 1.5% during the 5 years of life.

Furthermore, Ghana has gained strides in reducing its under 5-mortality rate at a steeper rate compared to the neonatal mortality rate [7], thus neonatal mortality still is a childhood problem.

Evidence also abound that diarrhea and respiratory infections are the commonest infections in LBWs. However, it is not well characterized what proportion of diarrheas in LBWs are due to RVGE, and what proportion of deaths from diarrheas are attributable to RVGE.

This study is therefore relevant to establish any such association with RVGE and birth weight in terms of morbidity and mortality. It will contribute to literature available on the association of RVGE and LBWs,, given the dearth of literature on this subject matter. The study will be relevant to inform policy and to focus policy on the most at risk population for RVGE. Thereby, reducing RVGE associated mortality in LBWs and thus contributing to overall reduction in LBW mortality. Furthermore, it can rekindle the need to up scale public health interventions that are capable of preventing low LBWs.

1.3 - OBJECTIVES

1.3.1 - GENERAL OBJECTIVES

To examine the relationship between birth weight and morbidity among infants with rotavirus gastroenteritis in a rotavirus vaccine trial in the Kassena-Nankana District, using secondary clinical trial data.

1.3.2 - SPECIFIC OBJECTIVES

1. To assess the relationship between birth weight and the time to onset of rotavirus gastroenteritis.
2. To assess the relationship between birth weight, and the incidence rate of rotavirus gastroenteritis in the first 2 years of life.
3. To assess the relationship between birth weight and the severity^s of diarrhea from rotavirus gastroenteritis.
4. To evaluate what proportion of diarrheas amongst LBWs are due to Rotavirus infection.

CHAPTER TWO

2.0 - LITERATURE REVIEW

2.1 - BURDEN OF RVGE

Globally, rotavirus accounted for about 2 million hospitalizations in children less than 5 years old [2]. From June 2006 to December 2008, WHO rotavirus surveillance in 18 sites of 13 African countries reported that 38% of hospitalizations for acute gastroenteritis were positive for rotavirus in under 5 year olds. Significantly, 90% of all rotavirus hospitalizations occurred in children between 3 - 12 months [8].

Eighty-two to eighty-five percent of RVGE mortality occurs in developing countries, mainly in South Asia and Sub-Saharan Africa [3, 6]. It is estimated that in 2008 alone about 453,000 children less than 5 year old died from RVGE [3]. This translates into the worrisome statistic of 1 in every 260 children born dying from RVGE, this figure corroborates mortality rates reported earlier by Parashar et al [2, 3]. RVGE accounts for 6% of diarrheal episodes and 20% of all diarrheal related deaths [2].

2.2 - CLINICAL MANIFESTATION OF RVGE

Clinically rotavirus presents with acute onset of fever in 50% of infected infants and non-bilious vomiting 80% of infected infants. This is usually followed by watery diarrhea for 24 to 72 hours after the onset of fever and vomiting in 65% of children [9, 10]. Dehydration and electrolyte imbalances are major sequel and cause of mortality especially in those who are between 2 – 24 months of age. Children with immunodeficiency such as LBWs [4, 11, 12], HIV infection, solid organ or bone marrow transplant or natural killer (NK) cell deficiency tend to have more severe

and prolonged diarrhea [11].

2.3 - BIRTH WEIGHT AND SUSCEPTIBILITY TO RVGE

Birth weight is an important determinant of infant morbidity and mortality [4]. The World Health Organization (WHO) defines LBW as “weight at birth less than 2500 g” [5], however favorable birth weight ranges from 3500 - 4500 g [4], has defined low birth weight (LBW). Globally about 15.5% of births are considered LBW thus about 20.6 million such infants are born each year [5]. 96.5% of them are in developing countries [4, 5]

LBW thus represents a diverse group of infants: some are premature (less than 37 completed gestational weeks), some are born at term but are small for gestational age (SGA) (weight for gestation less than 10th percentile), and some are both premature and SGA [5].

Being born with LBW is generally recognized as a disadvantage for the infant. LBW infants have higher risk for growth retardation, infectious disease, developmental delay and death during infancy and childhood. LBW infants suffer more deaths, hospitalizations, and diarrhea morbidity than normal BW infants. [4, 5]

Lira et al.(1996) in a prospective cohort study in Brazil determined that LBW were more likely to be sick longer with diarrhea and vomiting than normal BW infants [4]. They experienced 33% more days with diarrhea and 32% more days with vomiting than normal BWs [4]. Similar study by Das et al(1993) reported that diarrhea and upper respiratory infection occurred more often in full term intra-uterine growth restriction (IUGR) infants and premature infants than in normal BW infants [13].90% of the LBW deaths occurred in the post-neonatal period, the underlying causes

being diarrhea and respiratory infections [4]. LBW were 7 times likely to experience death from diarrhea and respiratory illnesses and 4 times likely to be hospitalized from diarrheal diseases compared to normal BWs during the first six month of life [4, 13].

Despite the adequate evidence from research about the burden of diarrhea in LBWs, there is paucity of data on what percentage of such diarrheas are caused by rotavirus as well as the incidence rate and severity of RVGE amongst LBWs.

2.4 - BIRTH WEIGHT AS A STATE OF IMMUNODEFICIENCY

Most LBWs are prematurely born hence, there is not adequate time in the womb for the organ systems; lungs, liver, digestive system, and other systems to mature and be fully functional at birth. Furthermore, the fetus gets antibodies from their mother across the placenta and this occurs mostly in the last 6-8 weeks of pregnancy [10, 14]. In a study by, Tammela et al., the IgG levels of the LBW infants were subnormal [12]. Thus premature birth deprives LBWs of this defensive resource. Poor immune status means susceptibility to common diarrhea infections including rotavirus infections and respiratory infections mainly [4, 14].

The WHO admonishes developing countries to substantially reduce their infant mortality rates by improving the care of LBW infants: through appropriate feeding, temperature maintenance, hygienic cord and skin care, and early detection and treatment of infections [5].

CHAPTER THREE

3.1 - METHODOLOGY - PRIMARY STUDY

This study was a secondary research that used secondary data. The data used for the analysis presented in this thesis was drawn from the clinical trial “Efficacy, Safety and Immunogenicity of RotaTeq™ Among Infants in Asia and Africa”.

3.1.1 - STUDY DESIGN

The primary study was a multi-center, double-blind (with sponsor blinding), placebo-controlled randomized trial. The trial was done to assess efficacy of three doses of pentavalent rotavirus vaccine against severe rotavirus gastroenteritis in infants. This was part of a multi-center trial conducted from April, 2007, to March, 2009, at medical facilities located in rural Kassena-Nankana district (Ghana); rural Karemo division, Siaya district, Nyanza province, western (Kenya); and urban Bamako (Mali). However the current research question was on the Ghanaian study.

3.1.2 - STUDY AREA

The primary study was conducted in the Kassena-Nankana District (KND) in Northern Ghana. It is one of the 245 districts in Ghana. It lies in the Guinea Savannah belt between 10°30' and 11°00' N, and 1.00' and 10°30'W. It borders Burkina Faso to the North. KND has an area of about 1675 km² and a population of 140,000. The Navrongo Demographic Surveillance System (NDSS) monitors the population dynamics of the area. The main occupation is subsistence farming, predominantly of millet and livestock. KND has a mean monthly temperature range of 20 – 45°C and rainfall

averages 800 – 1000 mm per annum, occurring mainly between May and October.

3.1.2 - PARTICIPANTS

Infants between 4 weeks and 12 weeks of age were eligible for enrollment in the study if they had no symptoms of active gastrointestinal disease and could be adequately followed up for safety by home visit or telephone contact (1 week and 2 weeks after any dose of vaccine or placebo) [1]. Breastfeeding was not restricted and there was no testing for HIV status. A total of 2227 children were recruited from the Kassena-Nankana district in the primary study.

3.1.3 - ADVERSE EVENTS

Study physicians reported and documented all serious adverse events occurring within 14 days of any dose and deaths or vaccine-related serious adverse events occurring at any time during the study. Serious adverse events were classified with the US regulatory definition, in line with ICH guidance, and identified by monthly query and parental reporting at any time or identification by study staff in hospitals or clinics. Intussusception at any time was also assessed. Home visits were attempted on days 3, 5, 7, 14, 21, and 42 after all vaccinations [1].

All adverse events were monitored by an independent, un-blinded data and safety monitoring board [1].

3.1.4 - DIARRHEA SURVEILLANCE

Surveillance was designed to identify severe gastroenteritis in participants on their presentation to medical facilities, with a common protocol in all study areas [1]. Infants who were enrolled in the

study were visited at least monthly to remind parents to bring their child to the clinic or hospital for symptoms of gastroenteritis.

Immune responses to vaccination were assessed in a subset of 450 participants . Venous blood was obtained immediately before the first dose of study vaccine or placebo was given, and 14 days after the third dose was given.

3.1.5 - ETHICS

The study protocol of the original study and consent forms were approved by the Western Institutional Review Board (Olympia, WA, USA), and institutional review boards and national ethical review committees for every site. Written informed consent was obtained from parents or guardians of all participants before enrollment. The study was done according to the principles of the Declaration of Helsinki and in compliance with good clinical practice guidelines [1].

3.1.6 - PROCEDURES

Infants were randomly assigned in a 1:1 ratio to receive three oral doses of pentavalent rotavirus vaccine 2 mL or placebo, at 6 weeks, 10 weeks, and 14 weeks of age. Doses were given with other routine pediatric vaccines, including oral poliovirus vaccine. Every dose of pentavalent rotavirus vaccine had an estimated potency of 2×10^7 infectious units per reassortant rotavirus in 2 mL of buffered liquid. Placebo contained the same constituents as the active vaccine but without viral antigens.

Rotavirus antigens in stool were detected by enzyme immunoassay. Wild-type rotavirus was confirmed by RT-PCR for identification of the VP6 genotype. Identification of rotavirus P and G

genotypes was done by RT-PCR[1].

3.1.7 - RANDOMIZATION AND MASKING

Unique allocation numbers were designated at Merck as pentavalent rotavirus vaccine or placebo with computer generated block randomization, with block sizes of six. Packages of vaccine and placebo were labelled with allocation numbers and provided to sites in identical presentations. Sites were instructed to assign allocation numbers to participants in sequential order as they were enrolled. Participants were enrolled by study staff, who remained masked to treatment assignment throughout the trial. In this double-blind trial, only the data and safety monitoring board and an associated Merck statistician, who was not involved in the rest of the trial, were unmasked to treatment assignment; the Merck statistician who analyzed the data, and the Merck and PATH protocol teams were masked to treatment assignment [1].

3.1.8 - PRIMARY AND SECONDARY OUTCOMES

The primary outcome was severe rotavirus gastroenteritis, irrespective of serotype, occurring 14 days or more after the third dose of vaccine or placebo, until end of study. Gastroenteritis was defined as three or more watery or looser-than-normal stools within a 24 hour period, or forceful vomiting. Stool samples were obtained from participants who presented to a medical facility. Medical staff took patient histories through interviews with the parent or guardian about symptoms of the present illness, and physical signs were documented by the staff who were caring for the participant. Data for symptoms and signs were obtained throughout the episode.

Severity of rotavirus gastroenteritis was defined by use of the 20-point modified Vesikari clinical scoring system, with a score of 11 or more classified as severe.

Secondary outcomes were vaccine efficacy against rotavirus gastroenteritis of any severity; disease scoring 15 or more or 19 or more with the Vesikari clinical scoring system; severe disease by individual serotype; severe disease between doses; severe gastroenteritis of any cause; and rotavirus gastroenteritis with a score of 17 or more with the 24-point Clark clinical scoring system. Post-hoc analyses were efficacy against severe rotavirus gastroenteritis occurring 14 days or more after the third dose in the first year of life, in the second year of life, and by country.

3.2 - METHODOLOGY - SECONDARY STUDY

The study was based on the study methodology and data collected from parent study as described above.

3.2.1 - PARTICIPANTS

Inclusion Criteria

1. For this study, all participants having data regarding their birth weight in the dataset.
2. All who satisfy 1 above and was randomized in the primary study was included in the analysis.
3. All who satisfy 1 and 2 above and had data regarding time of randomization and time to first RVGE was included in the analysis.

Exclusion Criteria

3. All participants with no data on birth weight.

3.2.3 - STUDY OUTCOMES

PRIMARY STUDY OUTCOME

1. Time to onset of first episode of diagnosed RVGE. **Diagnosed RVGE** was defined as an episode(s) of diarrhea or vomiting from whose stool any strain of RVGE has been identified by appropriate laboratory methods. **Entry date** was considered to be the time of randomization and **exit date** was considered to be 2 years post randomization for a specific participant as the primary study lasted 2 years. Participants were censored if they completed 2 years follow-up without developing RVGE, if death at any point during the study occurred or when lost to follow-up as a result of migration, voluntary or involuntary withdrawal on clinical grounds or withdrawal of the informed consent earlier signed. **Event occurrence** was identified as diagnosed first RVGE episode in a child.

SECONDARY STUDY OUTCOMES

1. Vesikari Clinical Score** was used to assess severity of RVGE.
** – see Appendix A for detailed explanation of Vesikari Clinical Score
2. Incidence rate of RVGE within the first 2 years of life expressed as person – time contribution to the trial.
3. Proportion of LBWs with diarrheas from which Rotavirus Antigen tested positive.

3.2.4 - DATA EXTRACTION & SYNTHESIS

The Department of Epidemiology in collaboration with the principal investigator for the primary clinical trial - Professor George Armah of Noguchi Memorial Research Institute, (cum my supervisor on this project) made available the primary data pertinent to answering the research

question.

The primary study data was available in SAS database format. The data was converted to Stata compatible datasets. The various datasets were browsed to understand its structure and needed variables pertinent to answering the various research questions above were identified. A datasheet was derived that contained all relevant variables pertinent to statistical analysis in the Stata 11TM statistical package. The following variables related to baseline data were extracted: Age at entry, Birth weight, Gender and whether participant was randomized to placebo or RotaTeq.

Another variable that categorizes the birth weight into LBW and NBWs based on WHO's definition was synthesized using appropriate Stata commands.

To determine the primary endpoint, the following variables were of interest: entry date that is the date of randomization, exit date which was the date of first AGE episode (caused by rotavirus, also for participants who were censored, exit date was the day on which censoring took place.

Variables used in determining the Vesikari Clinical Score as shown in **Appendix A** was extracted from the data, composite scores were then computed for each diarrheal episode.

The hospitalization date and discharge date as well were used to obtain duration of admission.

3.2.5 - STATISTICAL ANALYSIS

Statistical analysis was carried out using the Stata 11TM statistical package, and all analyses was done using appropriate Stata 11TM.

The data set was browsed and sorted looking for conflicting entries, mismatched variable types and missing entries. The analysis of the data set was by intention-to-treat and per-protocol. In the intention-to-treat analysis, missing data was included in the analysis.

As a rule, during hypothesis testing, the level of significance (α) was 0.05. P-values were for

two-tail tests generally. Confidence intervals were computed with 95% confidence levels.

3.2.5.1 - SAMPLE SIZE DETERMINATION

Binka et al, in 2003 estimated the cumulative incidence of rotavirus to be 39%[15]. Researchers assumed cumulative incidence rate of 39% for rotavirus gastroenteritis in the general children population. It was assumed that the incidence of rotavirus gastroenteritis will be about 10% more in low birth weight children, thus 49%. The power of the study was assumed to be 90% and the significance level 5%. The sample size powered adequately for this secondary study was 331 LBWs and 331 NBWs thus 662 in total.

3.2.5.2 - BASELINE CHARACTERISTICS

Children were stratified according to birth weight. The statistical analyses were done according to the birth weight categorization i.e. LBWs and NBWs based on WHO criteria - birth weight less than 2500 g is defined as LBW [16].

After assessing the distribution of variables, appropriate basic statistical summary measures were used. For instance, mean and standard deviation for continuous and normally distributed variables, median and interquartile range for skewed variables and proportions for categorical variables. Where necessary, corresponding 95% CIs was computed and additionally reported.

Baseline characteristics were compared between LBWs and NBWs. Baseline characteristics that were compared were age at recruitment, sex, proportion vaccinated and unvaccinated. Table 1 shows the baseline characteristics in both LBWs and NBWs. Any baseline characteristic, which was discordant, was treated as a covariate during adjusted analysis. See Appendix B - Table 1 dummy table.

3.2.5.3 - PRIMARY ENDPOINT ANALYSIS

The primary endpoint was time to first RVGE and was analyzed at two levels. The first level of analysis was the determination of time to first RVGE in children regardless of their vaccination status i.e. intention-to-treat analysis. The second level analysis was the determination of the primary endpoint of time to RVGE in the unvaccinated group only.

The primary endpoint of time to first RVGE was analyzed by time-to-event analytical methods i.e. Kaplan Meier and Cox regression methods. Children lost to follow-up due to migration, voluntary or involuntary withdrawal, death or those who completed the trial without mortality occurring were censored at the respective dates.

The overall median time to first RVGE and LBWs/NBWs specific median time to RVGEs was obtained and IQRs around the median was obtained as well.

Kaplan Meier curves were plotted for both LBWs and NBWs. The Log Rank Test used to compare the two curves obtained. Crude hazard ratios and corresponding 95% CIs were estimated using Cox regression model assuming proportional hazard ratios. The p-values for either the Wald statistic or the Likelihood Ratio test were reported.

Adjusted hazard ratios and corresponding 95% CIs were obtained for vaccinated and the unvaccinated group as well as baseline characteristics that were discordant between both LBWs and NBWs. For categorical variables with more than two options, the likelihood ratio test p-value was reported but for continuous or categorical variables with two variables, either the Wald statistic or likelihood ratio test p-value was reported.

Results from the analysis of the primary outcome were presented as tables (Tables 2-1 and 2-2 as shown in Appendix B).

3.2.5.4 - SECONDARY OUTCOME ANALYSIS

The crude incidence rate expressed, as person months for RVGE was obtained for all children included in the trial. Subgroup incidence rate for unvaccinated and vaccinated groups were and categorized by birth weight. Poisson regression model was used to determine the relative risk ratios and adjusted relative risk ratio.

The Vesikari Clinical Score (VCS) was computed using the indices extracted as variables from the dataset. See **Appendix A** for detailed Vesikari Clinical Scoring parameters. Composite Vesikari score was obtained for each child diagnosed with RVGE. An unpaired t-test was done to compare severity of RVGE for NBWs and LBWs..

See dummy tables for displaying the results in Appendix B - Table 3.

3.2.6 - ETHICAL CONSIDERATIONS

Approval Process: Ethical approval for the conduct of the secondary analysis was sought from Ghana Health Service Ethical Review Committee of the Research and Development Division of the Ghana Health Service.

Informed Consent Process: for the secondary research, the investigator relied on prior approved informed consent document and processes for the primary study. The informed consent included

prior permission for data collected to be used for further research purposes only such as this secondary research which will be using data from the primary research, without compromising confidentiality and exposing patients to undue risk from the loss of confidentiality.

Confidentiality: Patient's identities were concealed by way of using unique identifiers, which were a series of letters, numbers and symbols. Data was stored in a format that had adequate security features like access passwords to authenticate the person using the data, thus unauthorized persons were less likely to have access.

Benefits of this research: since this is a secondary research, there were no direct benefits to participants whose data were used. However, since RVGE and all-cause diarrhea has large morbidity and mortality burden in our society, the outcome of this study will be useful in stratifying and focusing care and future policy formulation.

Risks of this research to participant were minimal. There was minimal risk of loss of confidentiality but was curtailed by the use of the unique identifiers and not patient names.

3.2.7 - FUNDING

The investigator solely funded the research study, since it was an academic requirement towards attainment of an MSc degree in Clinical Trials.

Given the nature of the project, not much funding was required since investigator used secondary data from a completed clinical trial.

Incurred costs were mostly from printing, photocopying and using the Internet for journal search.

CHAPTER FOUR

4.0 - RESULTS

4.0.1 - INTRODUCTION

The results chapter presents the results following the statistical analysis as described in chapter 3 above. Baseline characteristics of participants are summarized in TABLE 1, and results for the primary and secondary endpoints in TABLE 2 and TABLE 3 respectively. TABLES 2 and 3 shows the crude and adjusted estimates of risk of developing rotavirus gastroenteritis by birth weight categorization, risk of occurrence of all cause diarrheas, incidence rate and relative risk of occurrence of rotavirus gastroenteritis and all caused diarrheas and finally the mean Vesikari Score compared using the student t test-statistic.

4.0.2 - BASELINE CHARACTERISTICS OF PARTICIPANTS

Of the 2,227 patients enrolled in the RotaTeq Navrongo Trial, 834 met the eligibility criteria representing 37.4%, which were the subgroup that were analyzed in this study. 1,393 enrolled children did not meet the threshold for inclusion into the analysis because there was no information on their birth weight in the dataset analyzed.

Children with birth weights less or equal to 2.5kg were considered Low Birth Weight (LBW) whilst children with birth weights greater than 2.5 were considered Normal Birth Weight (NBW). 137 / 834 had LBW thus 16.4% and 83.6% had NBW. The mean birthweight for the NBWs was 3.13 ± 0.38 kg whilst for LBWs was 2.31 ± 0.25 kg.

At baseline however, the two groups were balanced in terms of age at enrollment into the study, gender and randomization to either placebo or RotaTeq vaccine. The median follow-up for both

LBW and NBW was 527 days with inter-quartile range of 173 and 168 respectively (see Table 1 below).

Table 1: Baseline Characteristics

	NBW (n = 697)	LBW (n = 137)	TOTAL (n = 834)
<i>mean age – $\bar{x}(sd)$ → days</i>	58.9 (15.0)	58.0 (15.0)	58.7 (15.0)
<i>mean birthweight – $\bar{x}(sd)$ kg</i>	3.13 (0.38)	2.31 (0.25)	2.99 (0.47)
<i>Gender</i>			
<i>Male (%)</i>	346 (49.6%)	60 (43.8%)	406 (48.7%)
<i>Female (%)</i>	351 (50.4%)	77 (56.2%)	428 (51.3%)
<i>Vaccination status</i>			
<i>Unvaccinated(%)</i>	334 (47.9%)	74 (54.0%)	408 (48.9%)
<i>Vaccinated(%)</i>	363 (52.1%)	63 (46.0%)	426 (52.1%)
<i>Follow-up (med, IQR), days</i>	527 (168)	527 (173)	527 (182)

4.0.3 - PRIMARY ENDPOINT

A total of 47 / 697 (6.7%) reached the primary endpoint of RVGE in the NBW group compared to 7 / 137 (6.6%) for the LBW group. The median time to RVGE in days was 172 days (IQR=171) in the NBW group and 220 days (IQR=288) in the LBW group. See *Table 2* below.

Figure 1 below shows the Kaplan Meier plots of the cumulative probabilities of experiencing an RVGE from the time of enrollment. The two curves are superimposed closely on each other from the beginning of enrollment until about up to 160 days after enrollment where the two curves minimally diverge. About 550 days after follow-up, both groups tend to experience the event similarly until the trial ended.

Table 2. Results for the Primary Endpoint

Primary End Point	NBW (n = 697)	LBW (n = 137)	HR (95% CI)	P value
RVGE – n(%)	47 (6.7%)	9 (6.6%)	0.99 (0.49 - 2.04)	0.99 ^p
<i>Subgroup Analyses</i>				
<i>Unvaccinated^a</i>	31 (9.28%)	7 (9.46%)	1.04 (0.46 - 2.37)	0.92 ^p
<i>Vaccinated^b</i>	16 (4.41%)	2 (3.17%)	0.73 (0.17 - 3.18)	0.66 ^p
<i>Median time to RVGE – days, IQR</i>	172 (171)	220 (288)		

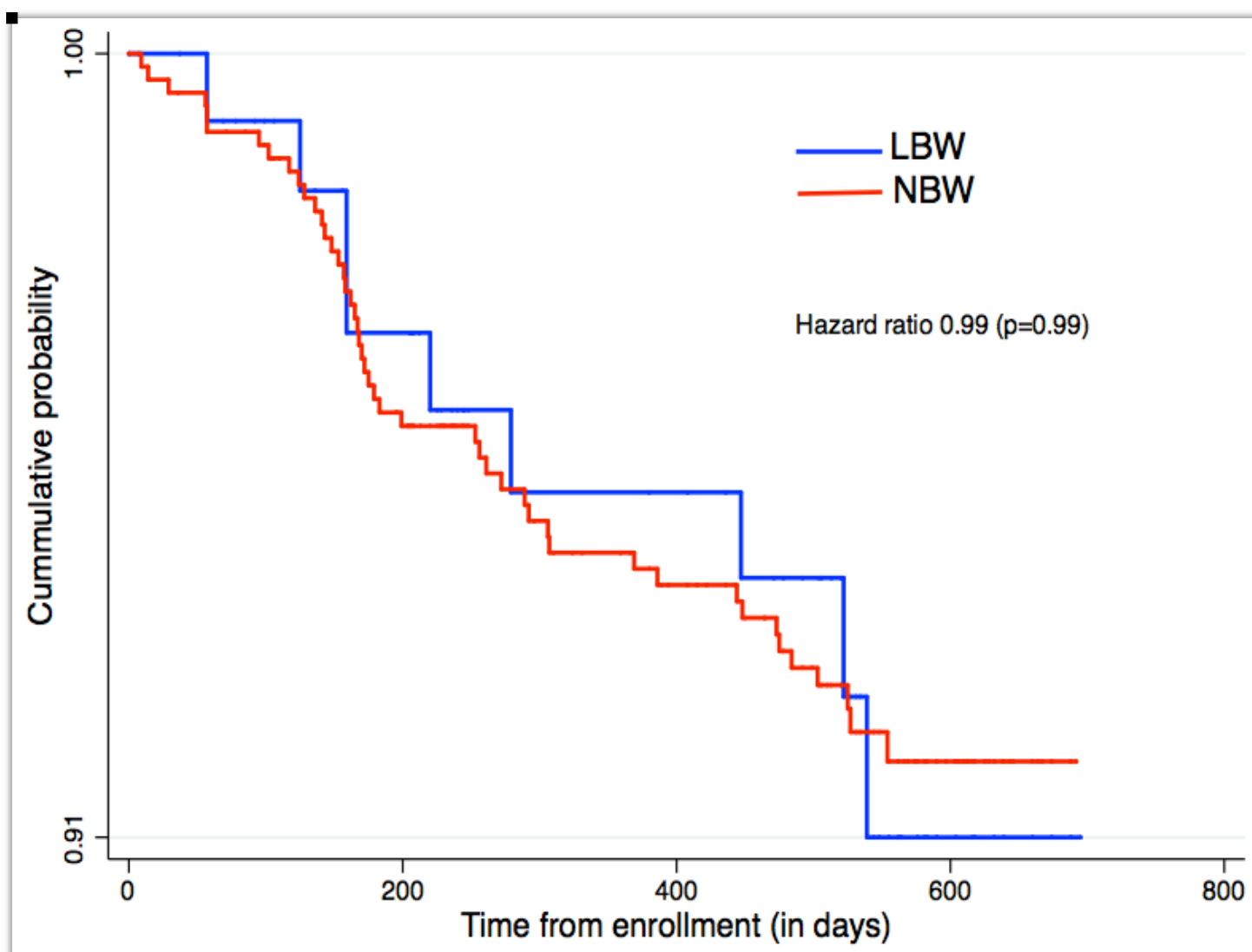
^p Wald's statistic p-value

^a received placebo during RotaTeq Trial

^b received Rotavirus vaccine during RotaTeq Trial

In a Cox regression analysis, the hazard ratio of contracting RVGE amongst the NBWs and LBWs was 0.99 (95% CI, 0.49 - 2.04). The Wald statistic and the Log Rank reported similar p-values of 0.99. Thus there was no significant difference in the risk of developing RVGE in NBW versus LBW groups for the duration of the trial.

FIGURE 1. - Kaplan Meier curve showing risk of RVGE by birth weight



In the subgroup analysis of the primary endpoint, **Figure 2.1** reveals the two curves are almost superimposed through out the duration of the trial in the vaccinated group with a *Log Rank* (0.01, 0.92), also the HR from **Table 2** shows no significant difference in time to RVGE in both LBWs and NBWs in the vaccinated group 0.73 (95% CI 0.17 - 3.18), p-value 0.66.

In **Figure 2.2** i.e the unvaccinated group, the survival curves were superimposed for the first 150 days, after which the LBW group were more at risk of developing RVGE until about 575 days where LBWs had survival advantage due to the crossing over of the curves. However, the *Log Rank* (0.18, 0.67) showed no difference in the general risk of developing RVGE irrespective of birth weight categorization. This is corroborated by the hazard ratio from Table 1 (HR 1.04, 95% CI 0.46 - 2.37).

FIGURE 2.1- Kaplan Meier curve showing risk of RVGE by birth weight in Unvaccinated Group

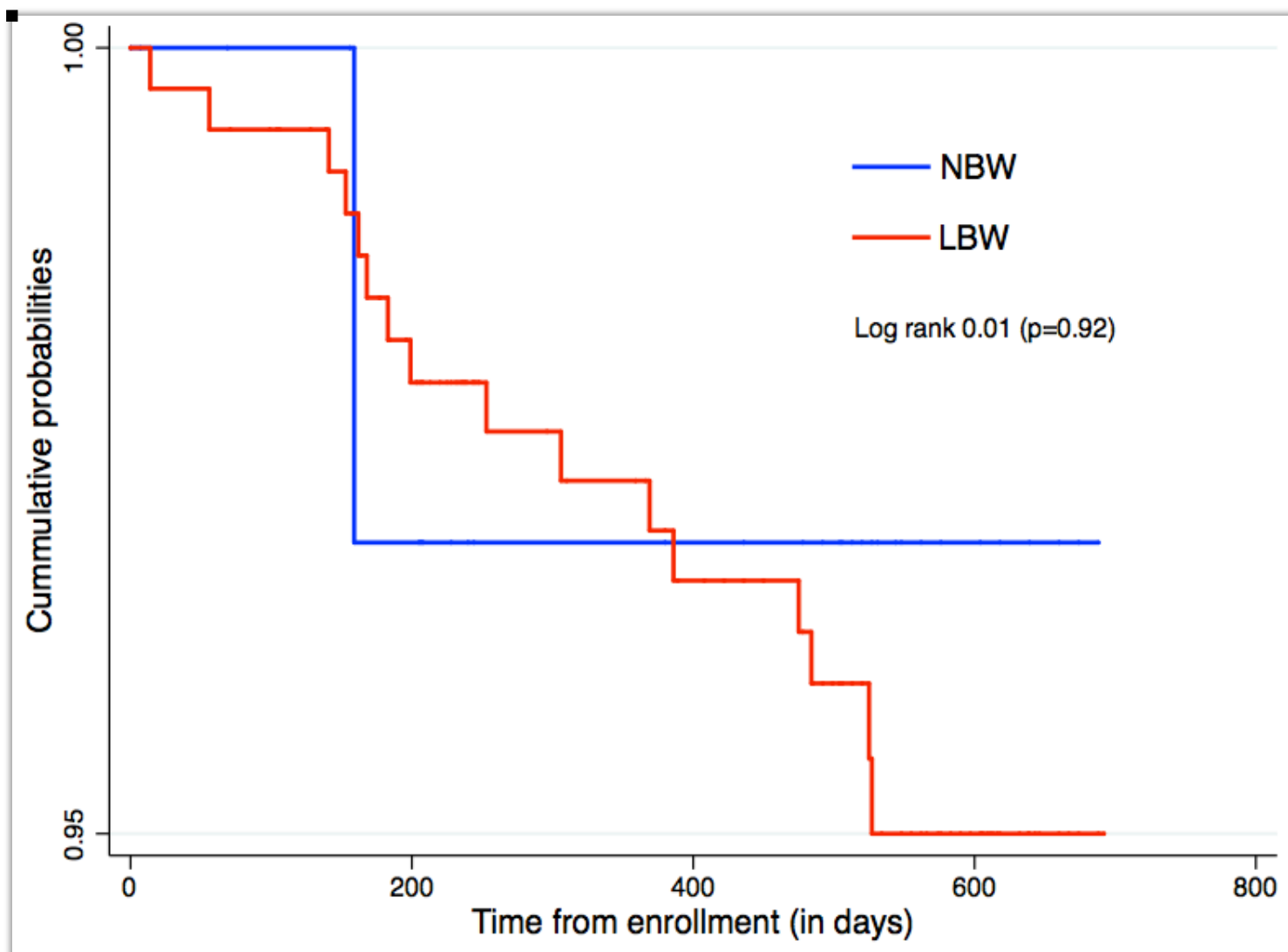
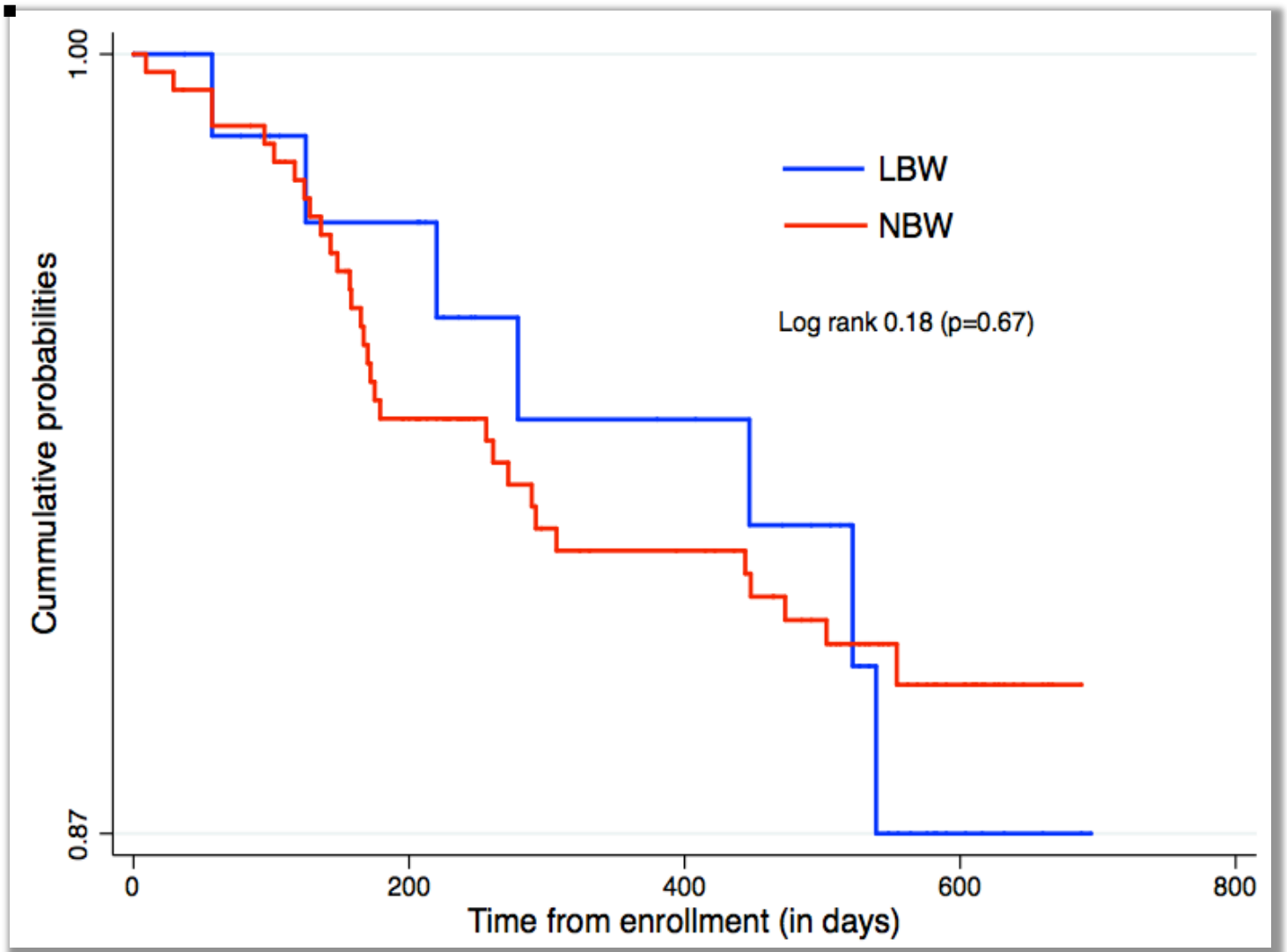


FIGURE 2.2 - Kaplan Meier curve showing risk of RVGE by birth weight in Unvaccinated Group



4.0.4 - SECONDARY ENDPOINT ANALYSIS

4.0.4.1 - INCIDENT RATE AND INCIDENT RATE RATIOS

Using Poisson regression model, the overall incidence rate per person days in the NBW group was 1.4 per 10,000 infant days and for the LBW group 1.39 per 10,000 infant days, which means the incidence rate of RVGE was comparable. The incidence rate ratio (IRR) obtained was 0.97 (95% CI 0.48 - 1.99) corresponding p-value is 0.94 suggestive of no difference in the rate of occurrence of RVGE between the two groups.

Table 3.1: Secondary Endpoint - Incidence Rate/Ratio

	NBW	LBW	IRR (95% CI)	p-val
<i>Crude IR (95% CI)</i>	1.40 per 10,000	1.39 per 10,000	0.97 (0.48 - 1.99) ^e	0.94 ^p
<i>Subgroup analyses</i>				
<i>Unvaccinated</i>	1.99 per 10,000	2.05 per 10,000	1.02 (0.45 - 2.30) ^e	0.96 ^p
<i>Vaccinated</i>	0.90 per 10,000	0.65 per 10,000	0.72 (0.17 - 3.13) ^e	0.66 ^p

^e - IRR - Poisson regression model

^p - Wald statistic associated with the Poisson regression model

IRR - Incidence rate ratio

IR - Incidence rate

For the subgroup analysis, the incidence rate in the unvaccinated group was 1.99 per 10,000 infant days in the NBW group and 2.05 per 10,000 infant days in the LBW group, with corresponding IRR 1.02 (95% CI 0.45 - 2.30) and p-value 0.96 suggesting there was similar risk of getting RVGE

in both NBW and LBW group during the trial period. As shown in Table 3.1 above, NBWs who were vaccinated had *0.90 per 10,000* infant days of new RVGE episodes as compared to *0.65 per 10,000* infant days in the LBW group who were vaccinated, however, per Poisson regression model, this difference in incidence rate was not significant as shown by IRR of *0.72 (95% CI 0.17 - 3.13)* with corresponding Wald statistic p-value of *0.66*.

4.0.4.2 - DIARRHEA SEVERITY USING VESIKARI SCORING SYSTEM

From Table 3.2 below, the mean Vesikari score for the 56 children diagnosed with RVGE was 8.45 ± 2.97 , thus moderately severe diarrhea is associated with RVGE in children less than 2 years old.

Analyzing the data by birthweight, LBWs had mean severity score of 9.22 ± 2.95 as compared to NBWs having a score of 8.30 ± 2.98 . Thus LBWs experienced more severe RVGE diarrheal episodes compared to NBWs, however the student's t-test p-value (0.40) used to compare the means showed no statistically significant difference.

The Vesikari scores were categorized into mild (< 7), moderate (7 - 10) and severe (≥ 11) and analyzed to demonstrate linearity. The Fisher's exact test p-values demonstrated no statistical significance in the severity of diarrhea amongst LBWs and NBWs, see Table 3.2. However, higher percentage of LBWs was noted to have more severe diarrhea than NBWs.

In the subgroup analyses dichotomizing the data by vaccination status did not affect the conclusion of no significance in the severity of RVGE in the crude or unsegregated analysis. Corresponding p-values, percentages and means in the subgroup analysis can be seen in Table 3.2 below.

Table 3.2: Secondary Endpoint - Vesikari Scores

	NBW	LBW	TOTAL	p-val
<i>mean(SD)</i>	8.30(2.98)	9.22(2.95)	8.45(2.97)	0.40 ^{tt}
CATEGORICAL				
<i>mild(%)</i>	13(27.6%)	1(11.2%)	14(25.0%)	0.50 ^{xx}
<i>moderate(%)</i>	24(51.0%)	5(55.6%)	29(51.8%)	
<i>severe(%)</i>	210(21.3%)	3(33.3%)	13(23.2%)	

Subgroup Analysis

Unvaccinated				
<i>mean(SD)</i>	8.32(3.47)	8.86(3.18)	8.42(3.38)	0.71 ^{tt}
CATEGORICAL				
<i>mild(%)</i>	11(35.5%)	1(14.3%)	12(31.6%)	0.58 ^{xx}
<i>moderate(%)</i>	12(38.7%)	4(57.1%)	16(42.1%)	
<i>severe(%)</i>	8(25.8%)	2(28.8%)	10(26.3%)	
Vaccinated				
<i>mean(SD)</i>	8.25(1.81)	10.5(2.12)	8.50(1.92)	0.12 ^{tt}
CATEGORICAL				
<i>mild(%)</i>	2(12.5%)	0(0.0%)	2(11.1%)	0.49 ^{xx}
<i>moderate(%)</i>	12(75.0%)	1(50.0%)	13(72.2%)	
<i>severe(%)</i>	2(12.5%)	1(50.0%)	3(16.7%)	

tt -- p-value from student's t test

xx -- p-values from Fisher's Exact Chi-Square test

CHAPTER FIVE

5.0 - DISCUSSION

5.1 - INTRODUCTION

This chapter synthesizes the findings of the results based on stated research questions and objectives. This study design is a cohort study nested within a randomized double blind placebo controlled trial that investigated efficacy of Rotavirus vaccine in Navrongo.

The discussion will be dichotomized to cover the risk of RVGE and birthweight and the burden or severity of RVGE versus birthweight in terms of the Vesikari scores obtained and incidence of RVGE.

The limitations of this study will also be discussed in this section.

5.2 - PREVALENCE OF RVGE

The prevalence of RVGE amongst the cohort of children under study was 22%. In 2003, Binka et al estimated the prevalence to be 39%[15]. This variation could be explained by the fact the cohort for this study included children who had received the rotavirus vaccination that conferred some protective effect. Evidently, the prevalence of RVGE was determined to be 28% for the unvaccinated group which correlates with the expected whilst that for RVGE amongst the vaccinated group was 15%.

5.3 - RISK OF RVGE BY BIRTHWEIGHT

The study results showed that the risk of RVGE is comparable in NBWs and LBW infants. This is based on the fact that 6.7% as compared to 6.6% of NBWs and LBWs from the cohort had RVGE diagnosed at the first diarrheal episode. Of NBWs and LBWs who had any diarrhea, 22.6% and 21.4% respectively were diagnosed RVGE. After thorough searches of PubMed, EMBase and MedLine, there were no research articles published that had done RVGE prevalence comparison along the lines of birth weight segregation.

A hazard ratio of 0.99 obtained with a corresponding p-value of 0.99 indicates no statistically significant difference between both infant groups regarding RVGE occurrence.

This finding does not corroborate the anchoring hypothesis that LBWs were more likely to be immunocompromised therefore were at higher risk RVGE compared with NBWs. Transplacental transfer of immunoglobulins G and protective Immunoglobulin A acquired through lactation engender carrier status of RVGE and prevents diarrheal episodes associated with rotavirus [17]. Mothers of children who formed the cohort in the population studied are known to provide breast-feeding exclusively in the first 6 months of life. This socio-cultural practice does allow for adequate transfer of protective antibodies thus making up for the immunologic deficits due to inadequate materno-fetal transfer trans-placentally in utero in LBWs. Therefore, susceptibility to RVGE in both LBWs and NBWs tend to be similar.

In the subgroup analyses, being vaccinated with Rotavirus vaccine or not did not appear to confound the risk of RVGE in the birthweight categories. In the unvaccinated subgroup LBWs had about 4% more risk in developing RVGE, however this difference in risk was not statistically significant as depicted by a p-value of 0.92. Contrarily, in the vaccinated subgroup, LBWs had 28% less risk of developing RVGE compared to NBWs. The differences in risk were shown not to be statistically significant. Thus being vaccinated or not did not affect the infant's risk for RVGE in the birthweight categories, nonetheless more benefit seemed to accrue to vaccinated LBWs cohort.

5.4 - BURDEN OF RVGE BY BIRTHWEIGHT

In the cohort studied for about 695 days, it was noted that the incidence of RVGE amongst LBWs was 1.39 per 10,000 infant days which is no different from 1.40 per 10,000 infant days for the NBW cohort. The mean birth weight of the low birth weight children enrolled was 2.31, which represents about 0.19 kg on average from the upper limit of LBW designation according to the criteria used. Thus indicating that children included in the LBW group were likely to be term born infants who experienced intrauterine

growth retardation before delivery hence was not deprived of placental transfer of antibodies that conferred protective effects. Another plausible explanation is the fact that the trial did not recruit severely premature infants who are expected to be deficient in maternal antibodies as a result of delivery before transfer occurred or had shorter exposure before delivery.

The mean Vesikari scores (which has been validated and) correlates with the severity of diarrhea, suggested that LBWs were likely to have higher scores than NBWs i.e. 9.22 versus 8.30. This difference was however possibly due to chance given the p-value was 0.40. Lira et al (1996), in their prospective cohort study in Brazil involving LBWs determined that LBWs developed severe forms of diarrhea [4], meeting threshold for statistical significance however, no such statistically significant difference was detected in our study. Suffice to mention that this study also looked at only RVGE as its endpoint and not all cause diarrhea as did Lira et al, hence the divergence. LBWs experienced 33% more days with diarrhea and 32% more days of vomiting than NBWs [4]. Das et al (1993) found that LBWs were 7 times more likely to experience death from diarrhea and respiratory illnesses and 4 times likely to be hospitalized from diarrheal diseases compared to normal BWs during the first six month of life [13]. Same as with the Lira et al study, this study used a combined endpoint of all cause diarrhea and respiratory illness, which departs from our study with the endpoint of RVGE. However, this study corroborates that finding in that, the proportion of moderate and severe RVGE based on the Vesikari score was higher in the LBW group than the NBW group.

5.5 - LIMITATIONS OF STUDY

Largely the primary data contained variables relevant to answering the primary question of the secondary analysis or study. According to sample size and power calculations in the protocol 331 were required in each arm. The primary data collected over data from over 2000 children and about 834 had data on birthweight, which may lead to selection bias. Another possible explanation could be that estimating the primary outcome for the primary study did not require using the birth weight variable hence incomplete data collection on that variable did not affect the primary and secondary objectives of the primary study. This is a recognized phenomenon, the primary data might not be based on the theory being used in the secondary analysis and the data may not reflect the requirements of the secondary analysis [18].

Also considering birth weight is a non-assignable characteristic, the primary study had an imbalance in the number of LBWs versus NBWs. Since the primary study did not start on the premise of collecting patient information based on stratifying them into the NBW and LBW arm the secondary analysis had an imbalance about 5:1 respectively. This represents unintended selective attrition. Baseline characteristics were comparable in terms of age at enrollment, gender, and vaccination status and follow-up duration and differed only in the numbers enrolled. Thus, this imbalance did not result in selective difference at baseline in the characteristics of the participants.

CHAPTER SIX

6.0 - CONCLUSION

Birth weight may not have an effect on the hazard of developing RVGE during the first two years of life. Thus low birth weight infants did not necessarily have an increased risk of developing RVGE within the first two years of life compared to normal birth weight infants.

The incidence rate of RVGE was similar amongst NBWs and LBWs within the first two years of life.

In terms of RVGE severity using Vesikari Score, LBWs experienced more severe episodes of RVGE than did NBWs but the trend did not reach statistical significance.

This research study may serve as a harbinger to definitively answering the question of the relationship between birth weight and RVGE.

Given that this was a secondary research study, a prospective cohort study will be appropriate to conclusively answer the question of whether birthweight will or will not affect the likelihood of developing RVGE in the first two years of life.

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APPENDIX A - VESIKARI SCORE

VESIKARI CLINICAL SCORING SYSTEM

Parameter	Score		
	1	2	3
<i>Diarrhea</i>			
Maximum Number Stools per Day	1-3	4-5	≥6
Diarrhea Duration (Days)	1-4	5	≥6
<i>Vomiting</i>			
Maximum Number Vomiting Episodes per Day	1	2-4	≥5
Vomiting Duration (Days)	1	2	≥3
Temperature	37.1-38.4	38.5-38.9	≥39.0
Dehydration	N/A	1-5%	≥6%
Treatment	Rehydration	Hospitalization	N/A

*Table adapted from rotavirus clinical trials utilizing the Vesikari clinical severity scoring system (Clark et al., 2004; Ruuska & Vesikari, 1990).

Severity Category			Maximum Score
<i>Mild</i>	<i>Moderate</i>	<i>Severe</i>	
<7	7-10	≥11	20

*Table adapted from rotavirus clinical trials utilizing the Vesikari clinical severity scoring system (Clark et al., 2004; Ruuska & Vesikari, 1990).

APPENDIX B - DUMMY TABLES**TABLE 1 - BASELINE CHARACTERISTICS**

Baseline Characteristics	NBW	LBW	TOTAL
<i>age(\bar{x},sd)---days</i>
<i>sex(%)</i>
<i>male(%)</i>
<i>female(%)</i>
<i>treatment</i>
<i>placebo(%)</i>
<i>RotaTeq(%)</i>
<i>follow-up time (median,IQR)–days</i>

TABLE 2-1 PRIMARY OUTCOME (UNADJUSTED)

VARIABLES	NBW (n)	LBW(n)	TOTAL (N)	p-value
<i>average BW –kg (\bar{x},sd)</i>
<i>% – diarrhoea</i>
<i>placebo</i>
<i>RotaTeq</i>
<i>% – RVGE</i>
<i>placebo</i>
<i>RotaTeq</i>
<i>median time to diarrhoea – days , IQR</i>
<i>placebo</i>
<i>RotaTeq</i>
<i>median time to RVGE – days , IQR</i>
<i>placebo</i>
<i>RotaTeq</i>
<i>CRUDE hazard – ratio , 95% – CI</i>

TABLE 2-2: ADJUSTED FOR COVARIATES (VACCINATION)

ADJUSTED HR	NBW (n)	LBW(n)	TOTAL(N)	p-value
<i>placebo</i>	(REF)	(REF)	(REF)	
<i>RotaTeq</i>
<i>sex</i>				
<i>female</i>	(REF)	(REF)	(REF)	
<i>male</i>

TABLE 3 - SECONDARY ANALYSIS FOR CRUDE AND ADJUSTED VALUES

VARIABLES	NBW (n)	LBW (n)	TOTAL(N)	p-value
<i>CRUDE – IR (95% – CI)</i>
<i>placebo</i>	(REF)	(REF)	(REF)	-
<i>RotaTeq</i>
<i>female</i>	(REF)	(REF)	(REF)	-
<i>male</i>
<i>CRUDE – IRR (95% – CI)</i>
<i>placebo</i>	(REF)	(REF)	(REF)	-
<i>RotaTeq</i>
<i>female</i>	(REF)	(REF)	(REF)	-
<i>male</i>
<i>VESIKARI – SCORE</i>				
<i>mean – VS, sd</i>
<i>placebo</i>
<i>RotaTeq</i>
<i>female</i>
<i>male</i>