Final Study Report for Non-Interventional Post-Authorisation Safety Study (PASS)

PASS Information

Title	A Study of the Effectiveness and Safety of a New Formulation of RotaTeq™ in Routine Use in a Developing World Setting
Version identifier of the final study report	Version 1.0
Date of last version of the final study report	August 31, 2018
EU PAS register number	8342
Active substance	RotaTeq™
Medicinal product	RotaTeq™ (Rotavirus Vaccine, Live, Oral, Pentavalent)
Product reference	N/A
Procedure number	N/A
Marketing authorisation holder(s)	Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc. 2000 Galloping Hill Road Kenilworth, NJ 07033, USA
Joint PASS	No
Research question and objectives	This non-interventional, prospective surveillance study collected data on the effectiveness and safety of the modified formulation of RotaTeq™ [RV5] (RV5mp) of in routine conditions of use in a developing-world setting.
Country(-ies) of study	Mali, West Africa

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1. ABSTRACT

Title: A Study of the Effectiveness and Safety of a New Formulation of RotaTeq™ in Routine Use in a Developing World Setting: First Annual Interim Report

Key words: RotaTeq™, rotavirus, effectiveness, safety, diarrhea

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Rationale and background

Rotavirus is the leading cause of severe diarrhea in infants and young children worldwide and is estimated to have caused 453,000 deaths or 37% of all deaths due to diarrhea among children less than 5 years of age worldwide in 2008 [1]. Most of the rotavirus gastroenteritis-related mortality occurs in developing countries where the access to treatment is suboptimal [2].

The current formulation of the pentavalent rotavirus vaccine (RV5: RotaTeq; rotavirus vaccine, live, oral, pentavalent), \RotaTeq™, must be stored refrigerated at 2 to 8°C. This presents challenges in countries where the ambient temperatures are high and the cold chain is not reliable. To better meet the needs of developing countries, a modified formulation of RV5 (RV5mp) has been developed [18]. Although the formulation continues to require

refrigerated storage, it is designed for increased stability in the event of temperature fluctuations. Moreover, the tubes will display indicators (vaccine vial monitors) that signal when a temperature deviation has occurred. The pre-licensure program will provide analytical data and immunogenicity data from developed countries as the basis for the approval of a) RV5mp formulation of RotaTeq™. The RV5mp is vaccine vial monitor compatible, with stability at 37°C for 7 days. This study provides post-licensure data from monitoring the effectiveness and safety of the RV5mp formulation in a developing-world setting.

This study was terminated early in October 2017 because the application for approval of the RV5mp formulation was withdrawn.

Research question and objectives

The objective of this non-interventional study is to monitor the effectiveness and safety of the RV5mp formulation among children who have been vaccinated with three doses of RotaTeq™ during routine conditions of use in a developing-world setting. The moderate to severe diarrhea (MSD) surveillance component of the study is designed to monitor the rate of rotavirus-positive diarrhea that occurs 14 or more days after vaccination with the third dose of RotaTeq™. Surveillance monitoring will occur in time periods before and after RV5mp vaccine introduction among children who are hospitalized or visit the urgent care department at selected surveillance sites in Mali. The intussusception surveillance component will perform active surveillance for intussusception among infants seeking care at the major referral hospitals in Bamako, Mali in order to estimate the incidence of intussusception among vaccinated infants before and after introduction of the RV5mp RotaTeq™ vaccine. These components of the study are descriptive and do not formally test hypotheses.

This report includes all study data, excluding a three-month run-in period during which case detection procedures were optimized. For both IS and AGE objectives, this includes cases enrolled from August 1, 2015 to September 28, 2017. This study included only children who had received the current formulation of RotaTeq™ because the RV5mp RotaTeq™ vaccine was never released.

Study design

This study does not involve active administration of RotaTeq™. All recipients of RotaTeq™ studied under this protocol received RotaTeq™ in the course of ordinary public health practice. The design is a non-interventional, prospective, pre-post surveillance study conducted in Bamako, Mali among patients seeking care at 6 referral centers and 3 hospitals for the IS component and at 9 health care facilities (including 6 peripheral health centers, 2 referral centers and 1 community hospital) for MSD component. The study is designed to conduct a descriptive comparison of rates of rotavirus MSD and intussusception among children vaccinated with the current formulation of RotaTeq™ (pre) to that of children vaccinated with the new formulation once it is available (post). All children enrolled in the

MSD component of this study provided a stool sample, which was tested for rotavirus antigen using Oxoid ProSpecT™ Rotavirus ELISA Kit (Basingstoke, United Kingdom).

Monitoring these rates will provide a descriptive assessment of the continued effectiveness and safety of the new formulation. Prior to the use of the RV5mp formulation, a health care facility-based surveillance system will be established utilizing a demographic surveillance network at selected health care facilities to detect cases of rotavirus MSD. In addition, cases of intussusception among infants vaccinated with the current formulation will be detected using a surveillance system, developed as part of this study, of selected referral centers and hospitals.

The DSS, comprised of two quartiers within Bamako, is the sampling frame and source of population-based census data for calculating rotavirus MSD rates for this study. Healthcare Utilization & Coverage Surveys (HUCS) are conducted twice a year in which age-stratified samples of children are randomly selected from the DSS population for surveys. The HUCS determines the proportion of children experiencing episodes of diarrhea overall, and episodes meeting the case definition of moderate-to-severe diarrhea during the previous week who visited a health center for care. The HUCS data will allow adjustment of the incidence of rotavirus derived from hospital surveillance to account for children with diarrhea who did not seek care. Vaccine coverage surveys are embedded within the DSS. These surveys include the number of doses of RotaTeq™ children received and at what time intervals. Vaccination cards will be reviewed, or, if no card is available, attempts will be made to verify the vaccination data with health center records.

The overall population of Bamako, based on city census data, is the source of data for calculating intussusception rates for this study. To determine vaccine coverage to use for intussusception data analysis, procedures for the estimation of rotavirus vaccine coverage will be developed by PPD investigators at the PPD. These procedures will include conducting coverage surveys to estimate rates of vaccine coverage in Bamako.

Setting

Sentinel health centers in Bamako, the capital city of Mali

Subjects and study size, including dropouts

1227 AGE cases were enrolled in this study, from 1 August 2015 to 28 September 2017.

Variables and data sources

The primary outcome variable for effectiveness includes moderate-to-severe rotavirus acute gastroenteritis (MSD) defined as: (1) an episode of three or more watery or looser-than-normal stools within the previous 24-hour period; (2) meets the case definition of moderate to- severe diarrhea (MSD); and (3) rotavirus detected in a stool specimen taken within 7 days after the onset of diarrhea. MSD is defined as: (1) diarrhea onset within 7 days of the health

care visit, beginning after 7 days without diarrhea, with the presence of one of the following: a) sunken eyes, more than usual; b) decreased skin turgor; c) IV rehydration recommended; or d) hospitalization recommended. To be considered as analyzable for effectiveness, cases must occur at least 14 days after dose 3 of RotaTeq™. The primary outcome variable for safety includes confirmed intussusception (by chart review, using the Brighton collaboration case definition and a panel of three adjudicators blinded to vaccination status) occurring in the 30 day period following any dose. The vaccination status of children enrolled in the study will be assessed as well as the population–based vaccination coverage of RotaTeq™ in the study area. Additional variables include clinical and demographic information, including age and gender of the enrolled child, parental socioeconomic status, age and number of siblings, medical history, subject-level and regional-level vaccination coverage and census-based population size of the study catchment area(s).

Results

After a three month run-in period, official study enrolment began on August 1, 2015 for both the MSD and intussusception studies. The study ended on 28 September 2017, when 1227 AGE cases had been enrolled and six rounds of HUCS surveys were carried out. During the nearly 26 months of enrolment, we observed an annual rotavirus-associated MSD incidence rate of 5.88 cases per 100 child-years among children 6-23 months of age who were fully vaccinated with RotaTeq (95% CI: 2.97 – 8.80). During intussusception surveillance, 83 suspected IS cases were enrolled and adjudicated. Of the 83 cases, 59 were confirmed as IS by the adjudication committee using the Brighton collaboration criteria. Based on the findings of the first Bamako-wide vaccine coverage survey, we estimated that the annual IS rate among children within one month after any dose of RotaTeq was 5.83 per 100,000 (95% CI: 0.55 – 11.212 per 100,000). The estimated incidence for IS among vaccinated children, regardless of time since last RotaTeq vaccination, was 30.68 per 100,000 (95% CI: 19.18 – 42.18 per 100,000), based on 57 cases and the estimated coverage rate in Mali of RotaTeq. The estimated incidence for IS among unvaccinated children was 39.99 per 100,000 (95% CI: 0 – 96.38 per 100,000), based on 2 cases and the estimated coverage rate in Mali of RotaTeq. The overall annual incidence of IS among all Bamako infants (whether vaccinated or unvaccinated) during our study period was 30.07 per 100,000 (95% CI: 19.00 – 41.14 per 100,000).

Discussion

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2. LIST OF ABBREVIATIONS

AC: Adjudication Committee
AE: Adverse Event
AGE: Acute Gastroenteritis
CRF: Case Report Form
PPD
DSS: Demographic Surveillance System
ENCePP: European Network of Centers in Pharmacoepidemiology and Pharmacovigilance
GEMS: Global Enteric Multicenter Study
GPP: Good Pharmacoepidemiology Practices
HUCS: Healthcare Utilization and Coverage Survey
IEC: International Ethics Committee
IRB: Institutional Review Board
IS: Intussusception
MAH: Marketing Authorization Holder
MSD: Moderate-to-Severe Diarrhea
RV: Rotavirus
RV5mp: modified formulation pentavalent rotavirus vaccine
SAE: Serious Adverse Event
SHC: Sentinel Health Center
SOP: Standard Operating Procedures
WHO: World Health Organization

3. INVESTIGATORS

Principal investigator	PPD USA
Coordinating investigator for each country in which the study is to be performed	PPD Mali
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4. MILESTONES

Milestone	Planned date	Actual date	Comments
Start of data collection for Intussusception	February 2015	May 6, 2015	
Start of data collection for MSD	February 2015	May 11, 2015	Start of the run-in period
End of run-in period	July 2015	July 2016	
End of data collection	June 2020	TBD	Dependent on introduction of RV5mp formulation in 2018

Milestone	Planned date	Actual date	Comments
Annual interim report 1 (Final Version)	December 2016	February 2017	Draft completed December 2016
Annual interim report 2	December 2017	Not planned	
Annual interim report 3	December 2018	Not planned	
Final Report	TBD	August 2018	
Close-out activities	TBD	September 2018	

5. RATIONALE AND BACKGROUND

General Background

This study will take place in Mali, a landlocked country in sub-Saharan West Africa (surface area ~1.2 million km^2), sharing borders with seven other African nations: Mauritania, Senegal, Guinea, Côte d'Ivoire, Burkina Faso, Niger, and Algeria. Mali has a population of ~14.5 million. Bamako, a malaria endemic area, is the capital and largest city, and home to ~12.4% of the population. The official national language is French, but this is largely the language of educated individuals (~70% of the population is illiterate). In practice, more than 40 tribal languages are spoken, although most Malians (~80%) can communicate in Bambara, the language of the most populous tribe. HIV prevalence among adults is low (~1%). In 2012, the United Nations ranked Mali as the 6th least developed country, with an under 5 mortality rate of 178 deaths per 1,000 live births, the 2nd highest rate in the world. Coverage with three doses of DPT vaccine is ~ 76%.

Mali has three main seasons (hot, hot rainy, and cool). The very hot month lasts from March to June; the hot, rainy period from June to October, and a cooler, dry period between November and March. Rotavirus exhibits a seasonal pattern in Bamako with peaks between July and February during the cool, dry months.

Rotavirus and Rotavirus in Mali

It is estimated that one third of all under-five diarrheal disease hospitalizations in Mali are caused by rotavirus. The Global Enteric Multicenter Study (GEMS) recently confirmed the public health burden of rotavirus in Mali, demonstrating that it is the most common cause of moderate-to-severe diarrhea in young children less than five years of age [4]. Studies in Africa show that the rotavirus vaccines are safe and effective against severe rotavirus disease and are cost-effective [5,6,7].

Study Implementation in Mali

The [PPD] Mali), located in [PPD] is the primary site for this study. [PPD] was established in 2001 by a formal agreement between [PPD] and the Malian Ministry of Health. [PPD] is a Clinical and Epidemiological Research Center with a dedicated scientific team of well-trained and highly experienced molecular biologists, microbiologists, epidemiologists, and physician scientists under the directorship of [PPD]. [PPD] has state-of-the art clinical and laboratory facilities and equipment, and is supported by an outstanding system of computers and information technology.

6. RESEARCH QUESTION AND OBJECTIVES

Rotavirus is the leading cause of severe diarrhea in infants and young children and is estimated to have caused 453,000 deaths or 37% of all deaths due to diarrhea among children less than 5 years of age worldwide in 2008 [1]. Most of the rotavirus gastroenteritis-related mortality occurs in developing countries where the access to treatment is suboptimal [2].

Since initial approval in the US in 2006, RotaTeq™ has been licensed in over 100 countries including countries in the European Union, North America, Latin America, Africa, and Asia.

A number of publications have documented a dramatic, consistent, and sustained reduction of rotavirus gastroenteritis after the introduction of rotavirus vaccine [3, 4, 5, 6, 7, 8, 9, 10, 11, 12]. In 2009, the World Health Organization recommended rotavirus vaccination be included in all national immunization programs to ensure access to rotavirus vaccines in the world's poorest countries [13].

The current formulation of RotaTeq™ must be stored refrigerated at 2 to 8°C. This presents challenges in countries where the ambient temperatures are high and the cold chain is not reliable. To better meet the needs of these countries, a new formulation of RotaTeq™ has been developed. The new formulation of RotaTeq™ is an enhanced thermostable formulation [18]. It has the same human-bovine rotavirus reassortants (G1, G2, G3, G4, and P1A[8]) as the current RotaTeq™, with only minor modifications to the buffer stabilizer [18].

There are no novel excipients in the new formulation. Importantly, the new formulation will meet the same minimum potency requirements for each serotype in addition to the maximum aggregate potency specification as the current formulation of RotaTeq™.

A clinical trial (V260-035) provided analytical data and immunogenicity data from developed countries as the basis for the approval of the RV5mp formulation of RotaTeq™. This study was designed to provide a post-licensure assessment of the effectiveness and safety of the RV5mp formulation in a developing-world. However, tthis study was terminated early in October 2017 because Merck elected to withdraw the pending U.S. supplemental Biologics License Application (sBLA) and EU Marketing Authorization Application (MAA) for RV5mp.

The research objectives are designed to describe and monitor trends of population-based incidence of rotavirus MSD and intussusception among children who are hospitalized or visit the urgent care department at selected health care facilities in Bamako, Mali. Therefore, these objectives are descriptive and do not formally test hypotheses.

First Primary Objective

Objective: (1) Objective: To compare rates of rotavirus MSD among children vaccinated with three
doses of the current formulation of RotaTeq™ to that of children vaccinated with three doses of the new formulation during routine use.

Second Primary Objective:

Objective: To compare rates of confirmed intussusception among children vaccinated with the current formulation of RotaTeq to that of children vaccinated with the new formulation during routine use.

Exploratory Objectives:

1. Objective: To assess the percent distribution of rotavirus G and P genotypes among rotavirus antigen-positive samples collected from children with MSD during the study surveillance period.
2. Objective: To estimate the coverage of the current and new RotaTeq vaccine formulation over the study period by conducting surveys among children in the catchment area.
3. To estimate the proportion of rotavirus MSD cases in children <5 years of age who utilize urgent care facilities or who are admitted to hospitals in the study area.
4. To characterize the clinical presentation and outcome of intussusception among enrolled subjects.
5. To characterize the clinical presentation and severity of MSD illness among enrolled subjects.

7. AMENDMENTS AND UPDATES

Number	Date	Section of study protocol	Amendment or update	Reason
2.0	03 November 2016	9.4, 9.5	Amendment	Clarified inclusion and exclusion criteria
2.1	03 May 2017	many	Update	Updated to the sponsor's new

Number	Date	Section of study protocol	Amendment or update	Reason
				protocol template

This study was terminated early in October 2017 because Merck elected to withdraw the pending U.S. supplemental Biologics License Application (sBLA) and EU Marketing Authorization Application (MAA) for RV5mp.

8. RESEARCH METHODS

8.1 Study Design

This study does not involve active administration of RotaTeq™. Mali introduced RotaTeq™ rotavirus vaccine in its national immunization program on January 15, 2014. All recipients of RotaTeq™ studied under this protocol received RotaTeq™ in the course of ordinary public health practice. The design is a non-interventional, prospective, pre-post surveillance study conducted in Mali within 6 referral health centers and 3 community hospitals for the IS component and at 9 health care facilities (including 6 peripheral health centers, 2 referral centers and a community hospital) for MSD component. Different centers were used for MSD and IS surveillance because the MSD study aimed to identify cases from two quartiers in Bamako, while the IS study aimed to identify all intussusception cases in Bamako. Additionally, because intussusception is a high-acuity condition, we expected that they would be detected from centers providing a more advanced level of care (such as hospitals and referral centers) rather community health centers. The study is designed to conduct a descriptive comparison of rates of rotavirus MSD and intussusception among children vaccinated with the current formulation of RotaTeq™ (pre) and children vaccinated with the new RV5mp formulation once it is available (post). Monitoring these rates will provide a descriptive assessment of the continued effectiveness and safety of the new formulation. Prior to the use of the RV5mp formulation, a health care facility-based surveillance system will be established utilizing a demographic surveillance system (DSS) and selected health care centers and hospitals to detect rotavirus MSD. In addition, cases of intussusception among infants vaccinated with the current formulation will be detected using a surveillance system of selected referral health centers and community hospitals.

The goal of the program is to establish a surveillance period of approximately two-years prior to the introduction of the RV5mp formulation. The surveillance for rotavirus MSD and intussusception among vaccinated children will then continue approximately two years after the introduction of the RV5mp formulation.

8.2 Setting

A PPD study team trained on this protocol conducts both the safety and effectiveness surveillance for this study within selected health care facilities. The selection criteria for these health care facilities for MSD surveillance included being part of an MSD surveillance effort in the PPD DSS area as part of GEMS. For the IS surveillance effort, most of the larger health care facilities in the capital city of Bamako that serve as referral centers for acutely ill children and/or have pediatric surgery facilities were considered for selection in the study. PPD personnel are responsible for collection of data required for the study, and either the laboratory capacity to perform rotavirus detection by the use of rapid antigen detection methods, or a reliable system for transporting specimens to a reference laboratory (e.g. PPD lab). These capabilities include the resources necessary for collecting stools appropriately and in a timely fashion, the storage of specimens in a refrigerator or freezer until testing is performed, personnel trained in testing, methods and adequate record-keeping practices in order to allow the coordination of laboratory and clinical data.

In 2006, PPD established a demographic surveillance system (DSS) in two quartiers (neighborhoods) in the capital city, Bamako: PPD and PPD Since performing an initial census on this subset of the Bamako population, all households have been visited 1-3 times annually to record births, deaths, migrations, and pregnancies. The population <5 years of age (~32,000 children) contributes to the denominator for calculating disease incidence for this study. Children who seek care for MSD at any of the 9 health centers or hospitals serving the DSS population (termed Sentinel Health Centers, or SHCs) are screened for rotavirus MSD. The SHCs include:

- PPD
- PPD
- 6 Community Health Centers in PPD

PPD

Surveillance for intussusception in this study is conducted using a network of 9 referral centers for acutely ill children and hospitals which serve as pediatric surgical centers within the capital city of Bamako, in order to capture cases for this relatively rare condition. These health centers include:

- PPD
- 2 other major hospitals in the city: PPD
- 6 District Health Centers

8.3 Subjects

8.3.1 MSD Surveillance Study

Approximately 32,000 children under five years of age reside in the demographic surveillance system (DSS) catchment areas of Bamako, the districts of [redacted] To be eligible for inclusion in the Moderate-to-Severe Diarrhea (MSD) surveillance study, subjects must meet the following inclusion criteria:

1) Less than five years of age at the time of the study visit

2) Parent(s)/Guardians(s) provide written informed consent for her/her child to participate in the study

3) Belongs to the DSS

4) Seeking care at a Sentinel Health Center (SHC) for diarrhea (three or more loose stools with the previous 24 hours) that has the following characteristics:

- New (onset after seven or more days diarrhea-free)
- Acute (onset in the previous seven days), and
- Meets at least one of the following criteria for MSD:

i. Sunken eyes (confirmed for the parent/caretaker as more than normal)

ii. Loss of skin turgor (abdominal skin pinch with slow (but less than two seconds) or very slow (greater than two seconds) recoil).

iii. Intravenous rehydration recommended; or

iv. Hospitalization recommended

Additionally, subjects enrolled in the MSD study may not meet the following exclusion criteria:

1) Subject diagnosed with dysentery

2) Any condition that, in the opinion of the investigator, might interfere with the evaluation of study objectives.

3) Enrollment in the study in the past 60 days

8.3.2 Intussusception (IS) Study

Recruitment for the IS surveillance study will occur from the entire city of Bamako, which includes approximately 71,699 children less than one year of age (infants). Children enrolled in the IS study will meet the following inclusion criteria:

1) Less than one year of age at the time of the study visit
2) Parents(s)/Guardian(s) provide written informed consent for his/her child to participate in the study.
3) Resides in Bamako
4) Hospitalized or has an urgent care visit for the treatment of clinician-suspected IS, per the case definition.

Children were excluded from the IS study for any condition that, in the opinion of the investigator, might interfere with the evaluation of the study objectives.

8.4 Variables

8.4.1 Exposures

8.4.1.1 Assessing exposure among individual enrolled cases of rotavirus MSD or suspected intussusception cases

8.4.1.2 Assessing vaccine coverage for the surveillance denominator for rotavirus MSD

8.4.1.3 Assessing vaccine coverage for the surveillance denominator for intussusception

8.4.1.4 Transitional period from current vaccine formulation to RV5mp formulation

It is expected that there will be a transitional period at the time when the RV5mp formulation is first introduced in Mali. Uptake of the RV5mp formulation will need to be monitored in the study area as a new birth cohort begins using the new formulation. PPD will remain in contact with the Ministry of Health to determine when the RV5mp formulation will be introduced and when the current formulation will be expended in Bamako. There will be a period where some currentformulation will still be in use – i.e., a transitional period as the old cohort completes vaccination with the current formulation and the new cohort starts the new formulation. The beginning of the RV5mp surveillance period will be associated with the birth cohort for which a high rate of RV5mp is administered in Bamako.

This transitional period never occurred because Merck elected to withdraw the pending U.S. supplemental Biologics License Application (sBLA) and EU Marketing Authorization

Application (MAA) for RV5mp. This report summarizes only data for the current formulation of RotaTeq.

8.4.2 Outcome

8.4.2.1 Rotavirus Case Definition

8.4.2.2 Rotavirus Severity

8.4.2.3 Intussusception Case Definition

8.4.2.4 Role of the Adjudication Committee (AC)

8.4.3 Covariates

Basic demographic information, including age and gender of the enrolled child, parental socioeconomic status, and age and number of siblings will be collected using a short questionnaire at enrollment by interviewing parents or from medical charts. Other information that could be relevant to rotavirus epidemiology, such as medical history, and vaccination history (confirmed using vaccination records), will also be collected at the time of enrollment. In addition to subject-level data on vaccination, data on regional and national RotaTeq™ vaccination coverage will be obtained based on procedures developed by PPD and described above.

8.5 Data sources and management

Data for this study will be obtained via primary data collection. The parent/guardian of the subjects enrolled in the study will provide information directly to the study team including subject demographics, vaccination history, feeding information, medical history, and current and prior medications. The study team will assess the clinical severity for all suspected MSD subjects according to a standardized scale. A whole stool sample will be collected from all subjects for rotavirus detection, and for PCR genotyping. Vaccination history will be recorded for all subjects. If parent/guardian is unable to provide vaccination card, the study team will attempt to verify health center records. For participants in the IS surveillance component, an adjudication committee will confirm cases of intussusception by reviewing available medical and surgical records.

The DSS described in Section 9.4 is the sampling frame and source of population-based census data for calculating rotavirus MSD rates for this study. The overall infant population of Bamako, based on city census data, is the source of data for calculating intussusception rates for this study. Healthcare Utilization & Coverage Surveys (HUCS) are conducted twice a year in which age-stratified samples of children are randomly selected from the DSS population for surveys. The HUCS determines the proportion of children experiencing episodes of diarrhea overall, and episodes meeting the case definition of moderate-to-severe diarrhea during the previous week who visited a health center for care. The HUCS data will allow adjustment of the incidence of rotavirus derived from hospital surveillance to account for children with diarrhea who did not seek care. Vaccine coverage surveys are embedded

within the DSS. These surveys include the number of doses of RotaTeq™ children received and at what time intervals. Vaccination cards will be reviewed, or, if no card is available, attempts will be made to verify the vaccination data with health center records.

8.5.1 Study Procedures

Refer to section 9.7 of the "A Study of the Effectiveness and Safety of a New Formulation of RotaTeq in Routine Use in a Developing World Setting" Protocol.

8.6 Bias

Because we are conducting active surveillance at sentinel health centers, it is possible that we will not ascertain all cases of MSD in our population. Potential cases may not present for care at a participating facility, or may not seek medical care at all. These cases may be different than cases that do present at health centers. Additionally, some parents will decline to allow their child to participate in the study—this may indicate wariness of medical interventions and programs, and may influence the probability that the child has received vaccines. As a result, selection bias may influence study findings.

In the intussusception study, it is possible that the effect of having more active intussusception case-finding by physicians will cause the rates of intussusception to rise over the course of the study period. Although this bias is difficult to control for, the use of a three-month intussusception surveillance run-in period will hopefully stabilize case-finding prior to the collection of study data to be used in analysis.

8.7 Study size

Because this is a surveillance study in which we will enroll all children who meet our case definitions, a sample size has not been calculated for this study. We anticipated that approximately 5000 children will be enrolled in either the MSD study or the IS study over the four year study period. In fact, 1473 children were enrolled in the study, including children enrolled during the run-in period.

MSD Study

Based on the experience of the GEMS study and the anticipated efficacy of the recently-introduced RotaTeq vaccine, it was planned in the protocol that about 1210 subjects with MSD would be enrolled each year, including 622 infants 6-11 months of age and 588 toddlers 12-23 months of age. During the study period of this report (August 1, 2015 – October 5, 2017), 688 children were eligible for inclusion in the first primary outcome analysis, being between 6 and 23 month of age and having been fully vaccinated with RotaTeq at least two weeks before the onset of diarrheal illness. Overall, 1227 children 0-59 months of age in total were enrolled for either the primary or exploratory objectives.

IS Study

About 160 children one year of age or younger were expected to be enrolled with suspected IS over the planned four years of study based on anticipated IS incidence. From August 1, 2015 – September 29, 2017, 83 suspected IS cases were included in the study. All of these cases have been reviewed by an adjudication committee, and their results are included in this report. Of the 83 possible IS cases, 59 were confirmed as intussusceptions, and 10 of these happened within 30 days of a dose of RotaTeq. No intussusceptions occurred after the first dose of RotaTeq, four of the IS occurred after the second dose of RotaTeq, and six occurred after the third dose of RotaTeq.

8.8 Data transformation

Children enrolled in the MSD study are described in 0-5, 6-11, 12-23, and 24-59 month age groups, as well as RotaTeq-eligible and RotaTeq-ineligible groups. RotaTeq-ineligible children are those who were too old to receive RotaTeq at the time it was introduced and those who were too young to have received a full course of RotaTeq. Children enrolled in the IS study are analyzed as a single age group.

8.8.1 Data management

Data collected for this study are typically collected on paper and entered into a secure online database. When internet connectivity at the sentinel health centre is strong enough, data may be entered directly into the database. The Emmes Corporation provides data management services using a SAS-based system, and provides SAS, STATA, CSV and SPSS datasets. Data have been analyzed in SAS and R.

8.9 Statistical methods

8.9.1 Main Summary Measures

This study will estimate rates of rotavirus MSD and intussusception pre and post the introduction of the RV5mp formulation. These analyses are descriptive and do not formally test hypotheses. Descriptive data analysis of the aggregated information will be performed and the results displayed in tabulated form. Descriptive statistics will be used to describe patient demographics and vaccine coverage and displayed in summary tables.

8.9.2 Main Statistical Methods

Refer to section 11 of the “A Study of the Effectiveness and Safety of a New Formulation of RotaTeq in Routine Use in a Developing World Setting" Protocol and the Statistical Analysis Plan for the same study. The first annual report will include only abbreviated analysis, focusing on descriptive analyses of the study participants and the outcomes ascertained to date.

8.9.3 Missing Values

Missing values will be denoted as missing in the close-out report’s descriptive tables.

8.9.4 Sensitivity Analyses

Not applicable

8.9.5 Amendments to the Statistical Analysis Plan

Not applicable

8.10 Quality Control

Quality control takes place at numerous points in the data collection cycle. Study case report forms (CRFs) are reviewed by either the PPD sponsored interviewers or lab technician and by a supervisor for completeness and accuracy. For all MSD CRFs (except for the strain-typing CRF), logical controls have been implemented in the electronic study database, managed by the Emmes Corporation. These controls prevent the primary entry of erroneous data. Additional quality checks are issued to the study team after data entry to ensure that missing questions and forms are completed in a timely manner. These CRFs are shared with a concurrent study, called "Rotavirus Vaccine Impact on Diarrhea in Africa" (VIDA), and the quality control and assurance measures undertaken are more thoroughly detailed in the VIDA Data Management Plan.

The IS CRFs and rotavirus strain-typing CRFs are scanned and stored as teleforms. After scanning but prior to entry into the database, all forms are quality-checked by the data management team to ensure that the forms are filled correctly and that the scanned entries are correctly interpreted. Additional details on the quality control of this database are available in the Teleforms Data Management Plan.

9. PROTECTION OF HUMAN SUBJECTS

For MSD, IS, HUCS and vaccine coverage survey study participants, consent by the child's guardian was documented with a date and signature on a consent form. The person conducting the consent discussion also signed the consent form. If the child's guardian was not able to read the consent form, an impartial third party who was literate witnessed the consent process and also signed the consent form. The protocol and consent forms for this project were approved by the PPD (IRB) and by PPD which serves as the ethical review board for PPD issued its approval on February 27, 2015, and PPD IRB issued its approval on March 4, 2015. Ethical approvals are reviewed annually at each site; the study was closed after enrolment ended in Mali on 29 September 2017. The study remains open at the PPD while data is being reviewed, and was last renewed on 13 December 2017.

10. RESULTS

10.1. **Primary objective #1**: To compare rates of rotavirus MSD among children vaccinated with three doses of the current formulation of RotaTeq to that of children vaccinated with three doses of the new formulation during routine use.

Participants

During both the run-in period and the main study period, 2361 children with diarrhea were screened for eligibility and 1380 children were enrolled based on study criteria. Of those who presented to sentinel health care centers after a three-month run-in period, 2112 children 0-59 months of age with diarrhea were screened for inclusion in the MSD study. Overall, 62.4% (1317/2112) of children who were screened for inclusion in the study were eligible for enrollment. Of those eligible, 93.2% (1227/1317) were enrolled in the study, although not all children contributed to all MSD study objectives. Children were recruited from 9 sentinel health centers in the PPD and PPD quartiers, the site of the PPD Demographic Surveillance System, from August 1, 2015 to September 29, 2017. Most (1130/1317, 85.8%) children were recruited out of the six community health centers. Table 1 shows the patterns of recruitment and enrollment of children from each center.

Table 1. Recruitment of eligible and enrolled children with MSD from sentinel health centers in PPD and PPD Bamako

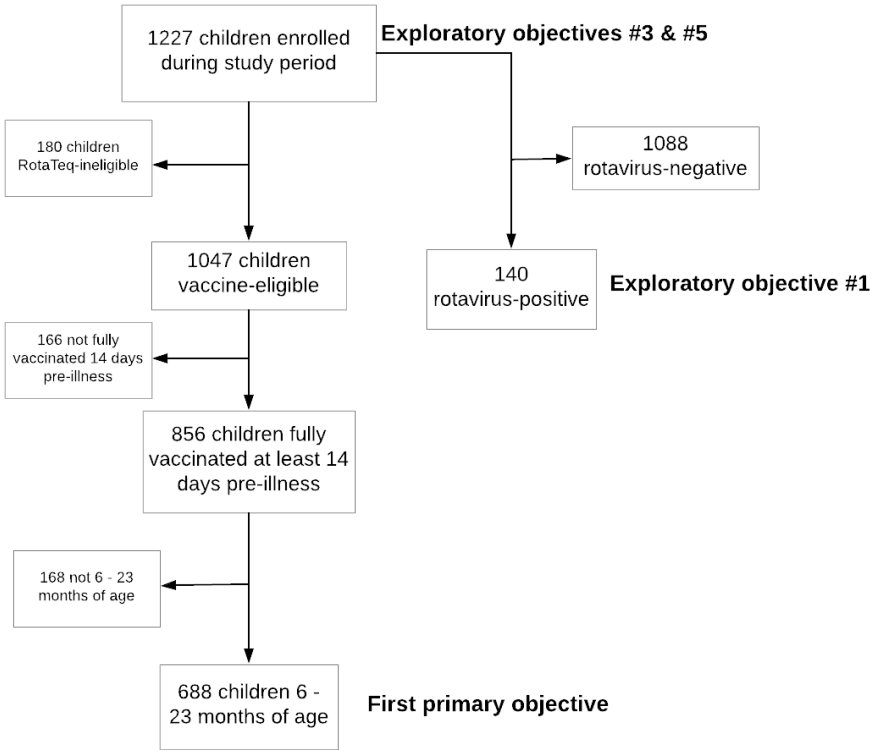
Center	# Eligible	# Enrolled	% eligible enrolled
PPD	57	36	63.2%
	382	329	86.1%
	16	16	100.0%
	49	48	98.0%
	289	281	97.2%
	47	45	95.7%
	245	244	99.6%
	1	1	100.0%
	231	227	98.3%
Total	1317	1227	93.2%

Most children who were eligible for the study were enrolled. However, 90 children who were eligible for enrollment were not enrolled in the study—most of these (80/90, 88.9%) were not enrolled because the child did not produce an adequate stool sample.

Figure 1 shows the derivation of MSD populations for the primary and exploratory study objective. Of the 1227 children enrolled, 85.3% (1047/1227) were RotaTeq-eligible, having been born after November 1, 2013. RotaTeq vaccination status was assessed by reviewing the child's vaccination card, reviewing the demographic surveillance system's vaccination records, or traveling to the EPI center where the child was vaccinated to review EPI records.

Based on the enrolled child's vaccination record, 81.8% (856/ 1047) of those vaccine-eligible had received their third dose of RotaTeq at least 14 days before the diarrheal episode began. 25 RotaTeq-eligible children (2.4%) did not have a vaccination record available to review. Most children (688 of 856) who were vaccinated with a complete series of RotaTeq were 6-23 months old—the age group that was both eligible to be fully vaccinated with RotaTeq and anticipated to be observable during the planned post- RV5mp formulation introduction years. These 688 children contribute to primary objective #1. It should be noted that Mali follows the WHO-recommended immunization schedule, which times administration of RotaTeq with other routine immunizations at 6, 10 and 14 weeks of age.

Figure 1. Enrollment in MSD protocol



Descriptive data

Data regarding the medical history, living conditions, economic status, physical examination, and vaccination history were collected during the enrollment study visit via a structured interview. Table 2 shows demographic features of the children with MSD who are included in this analysis. 49.7% (342/688) of fully vaccinated children were 6-11 months of age, while 50.3% (346/688) were 12-23 months.

Table 2. Primary Objective #1 population:
Children 6-23 months fully vaccinated ≥14 days pre-illness

	6-11 months, n = 342	12-23 months, n = 346	All ages, n = 688
# Female (%)	167 (48.8%)	161 (46.5%)	328 (47.7%)
# Mother Completing Primary Education (%)	87 (25.4%)	83 (24%)	170 (24.7%)
Median Household Size (range)	17 (3-89)	17 (3-73)	17 (3-89)
# With improved main water source[1] (%)	307 (89.8%)	312 (90.2%)	619 (90%)
# Living within 15 min walk of improved water source (%)*	170 (59.9%)	164 (59.2%)	334 (59.5%)
# Improved water available every day (%)	305 (89.2%)	309 (89.3%)	614 (89.2%)

[1]Improved water source defined as piped water, public tap, tubewell, covered well, protected spring, rainwater, or borehold
* 58 children 6-11 months and 69 children 12-23 months missing value

Outcome data

During the first year of surveillance, 98 cases of rotavirus-associated MSD were identified among the 688 fully vaccinated, enrolled children age 6 to 23 months (14.2% positive). Of those 98 cases, 21 were associated with severe rotavirus disease, defined as a modified Vesikari score of 11 or greater. Four rotavirus cases did not have a Vesikari score because information about receipt of intravenous rehydration was missing

Table 3. Primary objective outcome #1:
Rotavirus among fully vaccinated 6-23 month old children with MSD

Age group	Number of Cases	Number of Rotavirus Cases	Rotavirus Prevalence	Number of Severe Cases (Vesikari score ≥ 11)	Severe Rotavirus Prevalence
6-11 months	342	49	14.3%	11	22.9% (11/48)
12 - 23 months	346	49	14.3%	10	21.7% (10/46)
All ages	688	98	14.2%	21	22.3% (21/94)

Main results for primary objective #1

Because the RotaTeq modified process formulation was never introduced, we cannot fully evaluate the first primary objective to assess whether the incidence rate of MSD changes after the introduction of the new formulation. However, we can calculate the annual incidence of rotavirus MSD among children vaccinated with the current formulation of RotaTeq. The method used to calculate this parameter is shown in Annex 4.

The incidence of rotavirus in the fully vaccinated 6-23 month age group in the first year of the study is estimated at 5.88 per 100 child-years (95% CI: 2.97 – 8.80). The incidence rate among children 6-11 months of age was estimated at 7.60 per 100 child-years (95% CI: 1.68 – 13.51) and was estimated to be 5.07 (95% CI: 1.87 – 8.26) among children 12-23 months

of age. During the Global Enterics Multicenter Study (GEMS), which took place in Mali prior to the introduction of rotavirus vaccines, rotavirus MSD occurred among children 0-11 months of age at a rate of 8.4 (3.5-13.3) per 100 child-years and among children12-23 months of age at a rate of 4.1 (1.0-7.1) per 100 child-years.

10.2. **Primary objective #2:** To compare rates of confirmed intussusception among children vaccinated with the current formulation of RotaTeq to that of children vaccinated with the new formulation during routine use.

Participants

As with the AGE study, the start of intussusception surveillance was preceded by a three month run-in period. During this time, 42 potential participants were identified by chart review and physician report, and 10 potential IS cases were enrolled. Formal study enrollment ran from August 1, 2015 to September 29, 2017, during which time 359 children were identified who presented to health centers with signs and symptoms that met the surveillance definition of suspected IS. Of these 359, 344 children (95.8%) could be located for the study staff to obtain additional information about eligibility, and 194 (54.0%) of these were less than one year of age. Of the 194 case records suggesting that the child was age-eligible for the study, 10 were not enrolled because they were already enrolled in the study (i.e., they had sought care at more than one health center under surveillance), 2 were not enrolled because they did not meet IS criteria after further investigation, and 93 were not enrolled because they lived outside of Bamako. Of the 89 children remaining, 6 parents did not want the child to participate. In total, 83 children were enrolled in the formal IS study. Since some suspected IS cases could not be enrolled in the study (i.e., those whose parents did not agree to their enrollment, and those who could not be located), we expect that our rates are slight underestimates

As shown in Table 4, nearly all IS cases (95.2%) were identified at PPD. When cases were recognized at more than one health center as the child worked his or her way through the healthcare system, the child was enrolled out of the center where he or she received the most advanced care.

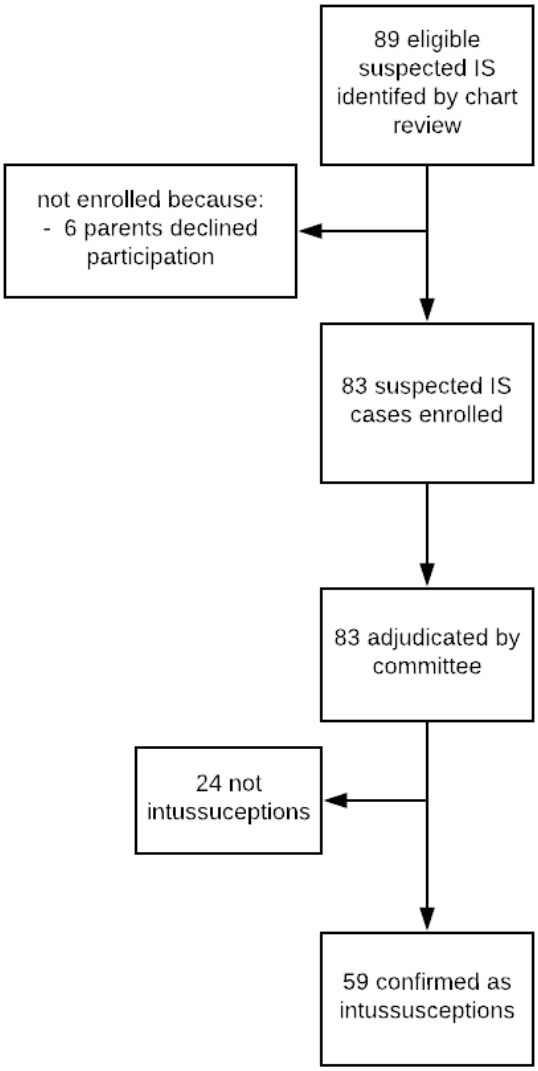
Table 4. Recruitment of suspected IS cases from sentinel health centers in PPD and PPD Bamako

Surveillance Site	Charts meeting surveillance definition	Able to locate child for more information	Age-eligible	Met IS enrollment criteria	Included
PPD	19	18	10	0	0
	21	21	7	1	1
	2	2	0	0	0
	6	3	4	0	0
	25	22	16	0	0

PPD	6	6	0	0	0
	254	252	153	85	79
	7	7	4	3	3
	17	13	11	0	0
Total	357	344	205	89	83

The consort diagram below (Fig. 2) shows the status of children enrolled in the IS study during the first year of enrollment, comprising the 83 enrolled children who represent the study population for this study's second primary objective and for exploratory objective #4.

Figure 2. Enrollment of children in the IS study



Descriptive data

Of the 83 suspected IS cases adjudicated during the study after the three-month run-in period, 36.1% (30/83) were female, all were younger than 12 months of age and 15 were less than one month of age. After adjudication, 71.1% (59/83) of cases were confirmed as IS (Table 5). None of the children less than one month of age were confirmed as intussusceptions by the adjudication committee. Ten children experienced IS within 30 days of receiving a dose of vaccine: none occurred after the first dose, 4 occurred after the second dose, and 6 occurred after the third dose (see Table 6).

Table 5. Suspected intussusception cases by age at enrollment

			Outcome of Adjudication	
Age in months	**Number enrolled**	**Number Female**	**Confirmed IS**	**Not IS**
0	15	8 (53%)	0 (0%)	15 (100%)
1	1	0 (0%)	1 (100%)	0 (0%)
2	5	1 (20%)	2 (40%)	3 (60%)
3	5	0 (0%)	3 (60%)	2 (40%)
4	4	1 (25%)	3 (75%)	1 (25%)
5	13	5 (39%)	13 (100%)	0 (0%)
6	9	5 (56%)	9 (100%)	0 (0%)
7	11	4 (36%)	10 (91%)	1 (9%)
8	8	3 (38%)	8 (100%)	0 (0%)
9	6	0 (0%)	6 (100%)	0 (0%)
10	6	3 (50%)	4 (67%)	2 (33%)
11	0	-	-	-
All	83	30 (36%)	59 (71%)	24 (29%)

Outcome data

Of the 59 cases with confirmed intussusception, 35.6% (21/59) were female. All but two confirmed cases had received at least one dose of RotaTeq; 10 had received a dose within 30 days of the intussusception; 5 children were 1-4 months of age and 5 were 5 months of age. Table 6 shows the breakout of RotaTeq doses received by confirmed IS cases, regardless of time elapsed since the last dose, and the number of intussusception cases occurring within 30 days of a dose, regardless of dose.

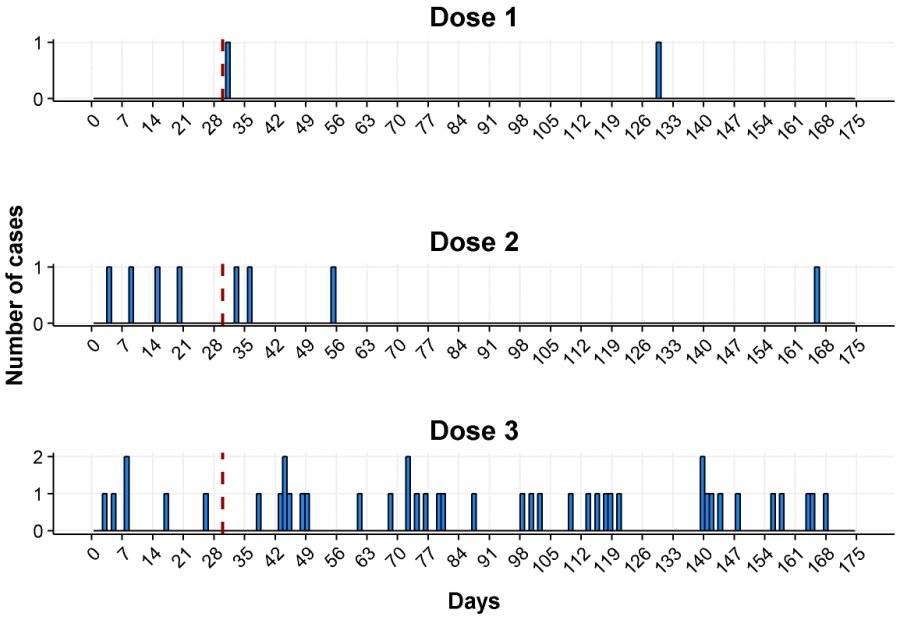
Table 6. RotaTeq exposure among 59 children with adjudication committee-confirmed IS

			Doses of RotaTeq Administered				
Age in months	**Number Confirmed**	**Number Female**	**0 doses**	**1 dose**	**2 doses**	**3 doses**	**Vaccinated <30 days**

	IS						before event
0	0	-	-	-	-	-	-
1	1	0 (0%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
2	2	0 (0%)	0 (0%)	1 (50%)	1 (50%)	0 (0%)	1 (50%)
3	3	0 (0%)	0 (0%)	0 (0%)	2 (66.7%)	1 (33.3%)	3 (100%)
4	3	1 (33.3%)	0 (0%)	0 (0%)	2 (66.7%)	1 (33.3%)	1 (33.3%)
5	13	5 (38.5%)	0 (0%)	1 (7.7%)	2 (15.4%)	10 (76.9%)	5 (38.5%)
6	9	5 (55.6%)	0 (0%)	0 (0%)	0 (0%)	9 (100%)	0 (0%)
7	10	4 (40%)	1 (10%)	0 (0%)	0 (0%)	9 (90%)	0 (0%)
8	8	3 (37.5%)	0 (0%)	0 (0%)	1 (12.5%)	7 (87.5%)	0 (0%)
9	6	0 (0%)	0 (0%)	0 (0%)	1 (16.7%)	5 (83.3%)	0 (0%)
10	4	3 (75%)	0 (0%)	0 (0%)	0 (0%)	4 (100%)	0 (0%)
11	0	-	-	-	-	-	-
All	59	21 (35.6%)	2 (3.4%)	2 (3.4%)	9 (15.3%)	46 (62.7%)	10 (16.9%)

Figure 3 shows the most recent dose and elapsed time since vaccination for confirmed IS cases who received a dose of RotaTeq. In total, 10 intussusception cases occurred within 30 days of a dose of RotaTeq. No IS cases occurred within 30 days after the first dose. Four of the IS cases occurredwithin 30 days of a second RotaTeq dose and six IS cases occurred within 30 days after a third RotaTeq dose.. Time elapsed between dose and an IS event ranged from 3 to 26 days. A red, dashed line marks 30 days.

Figure 3. Distribution of 59 confirmed IS cases by days elapsed since most recent RotaTeq dose (red dashed line indicates 30 days after a dose)



(n=59)

Main results for primary objective #2

Because the new formulation of RotaTeq was never released in Mali, it is not possible to carry out the comparison proposed in this objective. Additionally, because 25 months of IS cases had been observed prior to the writing of this closeout report, we have annualized this data.

The incidence of IS within 30 days of vaccination was estimated by:

$$\frac{m_1}{N_1} + \frac{m_2}{N_2} + \frac{m_3}{N_3}$$

where m_1, m_2, and m_3 are the number of children who experience an episode of IS within 30 days of their first, second and third dose, respectively, and N_1, N_2, N_3 are the estimated numbers of children in Bamako who have been vaccinated with exactly one, exactly two, and exactly three doses of RotaTeq. Denominators for this estimate were calculated as described in Annex 2. Over the study period, the annual rate of IS within 30 days of vaccination is estimated to be 5.83 per 100,000, based on 10 observed cases (95% CI: 0.55 – 11.12 per 100,000). The estimated incidence for IS among vaccinated children, regardless of time since last RotaTeq vaccination, is 30.68 per 100,000 (95% CI: 19.18 – 42.18 per 100,000), based on 57 cases and the estimated coverage rate in Mali of RotaTeq. The estimated incidence for IS among unvaccinated children was 39.99 per 100,000 (95% CI: 0 – 96.38 per 100,000), based on 2 cases and the estimated coverage rate in Mali of RotaTeq. Based on 59

cases and the entire birth cohort in Bamako of 94,179 infants as described in Annex 2, the annual incidence of IS among all Bamako infants (i.e. less than 12 months of age and regardless of vaccination status) is 30.07 per 100,000 (95% CI: 19.00 – 41.14 per 100,000).

10.3. **Exploratory objective #1:** To assess the percent distribution of rotavirus G and P genotypes among rotavirus antigen-positive samples collected from children with MSD during the study surveillance period.

Participants

All MSD cases under five years of age who were positive for rotavirus by EIA are included in the analysis for this objective. EIA results were verified at the PPD and genotypes were determined by sequencing. This included 140 children in total: 10 who were 0-5 months old, 54 who were 6-11 months old, 58 who were 12-23 months old, and 17 who were 24-59 months old.

Descriptive data

For exploratory objective #1, all enrolled children with EIA-positive rotavirus will contribute to the analysis. In addition to characterizing overall rotavirus genotype, children will be analyzed in 0-5, 6-11, 12-23 and 24-59 month age groups and by vaccination status as numbers of enrollees allows.

Table 7. Exploratory Objective #1 population: Rotavirus-positive children younger than 59 months of age

	0-5 months, n = 10	6-11 months, n = 54	12-23 months, n = 59	24-59 months, n = 17	All ages, n = 139
# Female (%)	3 (30%)	23 (42.6%)	26 (44.1%)	10 (58.8%)	62 (44.3%)
# Mother completing primary education (%)	4 (40%)	14 (25.9%)	16 (27.1%)	5 (29.4%)	39 (27.9%)
Median Household Size (range)	12 (4-25)	19 (3-89)	22 (3-65)	7 (3-36)	19 (3-89)
# Fully vaccinated with RotaTeq (%)	2 (20%)	49 (92.5%)	49 (86%)	7 (46.7%)	107 (79.3%)
# with improved main water source[1] (%)	9 (90%)	47 (87%)	52 (88.1%)	16 (94.1%)	124 (88.6%)
# within 15 min walk from improved water source* (%)	6 (66.7%)	28 (59.6%)	32 (69.6%)	7 (70%)	73 (65.2%)
# improved water available every day (%)	9 (90%)	47 (87%)	52 (88.1%)	15 (88.2%)	123 (87.9%)

1 Improved water source defined as piped water, public tap, tubewell, covered well, protected spring, rainwater, or borehole
*1 child 0-5 months, 7 children 6-11 months, 13 children 12-23 months and 7 children 24-59 months (28 overall) missing value

Outcome data

At the writing of this report, 140 of the 140 rotavirus-positive samples isolated during the main study enrolment period had been fully genotyped.

Main results for exploratory objective #1

70% of the 140 rotavirus-positive samples typed to G1P[8] human rotavirus, 14% typed to G1P[6] human rotavirus, 7% typed to G6[P6] human rotavirus, and 1% typed to G2P[4] human rotavirus. 7% of specimens could not be fully typed, and 1 specimen did not test positive for rotavirus on repeat EIA. These results are shown in Table 8.

Table 8. Distribution of rotavirus strains

	Vaccination status			
Strain	**None/incomplete**	**Complete**	**Unknown**	**All**
G1P[8] human	20 (71%)	74 (69%)	4 (80%)	98 (70%)
G1P[6] human	5 (18%)	14 (13%)	1 (20%)	20 (14.3%)
G6P[6] human	2 (7%)	8 (7%)	0 (0%)	10 (7.1%)
G2P[4] human	0 (0%)	1 (1%)	0 (0%)	1 (0.7%)
G1P[X] human	1 (4%)	2 (2%)	0 (0%)	3 (2.1%)
G6P[X] human	0 (0%)	1 (1%)	0 (0%)	1 (0.7%)
GXP[6] human	0 (0%)	1 (1%)	0 (0%)	1 (0.7%)
Genotype not determined	0 (0%)	5 (5%)	0 (0%)	5 (3.6%)
Rotavirus negative on repeated ELISA	0 (0%)	1 (1%)	0 (0%)	1 (0.7%)

10.4. **Exploratory objective #2:** To estimate the coverage of the current and new RotaTeq vaccine formulation over the study period by conducting surveys among children in the catchment area

Participants

The children included in this objective are those enrolled in one of the six HUCS rounds conducted during data collection. The demographics and details of these HUCS rounds are included in Appendix 3. In total, 11895 children were selected to be surveyed and 10078 children participated in a survey during this study.

Descriptive data

Apart from age, which is detailed in Appendix 3, no covariates were used in estimating RotaTeq coverage.

Outcome data

Table 9 shows RotaTeq coverage by dose in the six combined HUCS surveys among children eligible to have received RotaTeq (born on or after November 1, 2013). In the 6-11 month age group, 87% of children had received three doses. Among the 6-23 month age, 87% of children whose vaccination records could be reviewed had received three doses. Results weighted by the fraction of children sampled in each age group during each survey round are shown in Table 10; the results below (Table 9) are not weighted.

Table 9. Exact number of RotaTeq Doses by age among HUCS enrollees (Six surveys conducted in period June 15, 2015 – February 12, 2018)

			Exact number of Doses			
Age group	**# with vaccination record**	**# missing vaccination record**	**0**	**1**	**2**	**3**
0-5 months	575	35	16 (3%)	53 (9%)	117 (20%)	389 (68%)
6-11 months	2721	208	87 (3%)	92 (3%)	186 (7%)	2356 (87%)
12-23 months	2704	422	126 (5%)	93 (3%)	136 (5%)	2349 (87%)
24-59 months	677	294	32 (5%)	27 (4%)	36 (5%)	582 (86%)
6-23 months	5425	630	213 (4%)	185 (3%)	322 (6%)	4705 (87%)

Main results for exploratory objective # 2

Exploratory objective #2 cannot be fully assessed because the new RotaTeq vaccine will not be distributed. The weighted estimates of RotaTeq coverage are shown below in Table 10. RotaTeq coverage is defined as the proportion of children having received three doses of RotaTeq.

Table 10. Three-dose RotaTeq coverage among children residing in PPD and PPD DSS

Age Group	Estimated coverage	Lower 95% CI	Upper 95% CI
0 - 5 months	67.2%	62.3%	72.0%
6 - 11 months	86.8%	85.7%	87.9%
12 - 23 months	85.5%	84.3%	86.8%
24 - 59 months	31.6%	30.0%	33.3%
6 - 23 months	86.0%	85.1%	86.8%

10.5. **Exploratory objective #3:** To estimate the proportion of rotavirus MSD cases in children <5 years of age who utilize urgent care facilities or who are admitted to hospitals in the study area.

Participants

All enrolled 0-59 month old children meeting the surveillance definition for moderate-to-severe diarrhea are included in the analysis for this objective. Of those enrolled 100 (8.1%) were 0 to 5 months old, 378 (30.8%) were 6 to 11 months old, 425 (34.6%) were 12 to 23 months old and 324 (26.4%) were 24 to 59 months old. 1227 children in total are included in this analysis.

Descriptive data

For this objective, children will be analyzed in 0-5, 6-11, 12-23 and 24-59 month age groups because we expect that the proportion of cases with rotavirus and the clinical presentation of MSD will differ by age.

Outcome data

The proportion of MSD cases associated with rotavirus by age group is shown in Table 11. Unlike the population derived for the first primary objective, this objective includes both children who have been vaccinated with RotaTeq and those who have not. All children had rotavirus EIA results available.

Table 11. Rotavirus positivity among cases of moderate-to-severe diarrhea, by RotaTeq status

		# (%) Rotavirus Positive			
Age Group	# Enrolled	Not vaccinated with RotaTeq	Partially vaccinated with RotaTeq	Fully Vaccinated with RotaTeq	All
0-5 months	100	3/8 (37.5)	4/39 (10.3)	3/53 (5.7)	10 (10.0%)
6-11 months	378	1/10 (10)	3/21 (14.3)	49/345 (14.2)	54 (14.3%)
12 - 23 months	425	5/26 (19.2)	2/34 (5.9)	50/349 (14.3)	59 (13.9%)
24-59 months	324	6/124 (4.8)	2/30 (6.7)	7/147 (4.8)	17 (5.2%)
		15/168 (8.9)	11/124 (8.9)	109/894 (12.2)	
0-59 months	1227	3/8 (37.5)	4/39 (10.3)	3/53 (5.7)	140 (11.4%)

Main results for exploratory objective #3

Among all enrolled children 0-59 months presenting to sentinel health centers with moderate-to-severe diarrhea, 11.3% were rotavirus positive. As shown in Table 12, the greatest burden of rotavirus is present in the first two years of life, where rotavirus is associated with over 13% of MSD episodes.

Table 12. Rotavirus positivity among children with MSD at sentinel health centers

Age Group	% Rotavirus Positive
0-5 months	10.0%
6-11 months	14.3%
12 - 23 months	13.9%
24-59 months	5.2%
0-59 months	11.4%

10.6. **Exploratory objective #4:** To characterize the clinical presentation and outcome of intussusception among enrolled subjects.

Participants

Participants include the 59 confirmed intussusception cases described in Primary Objective #2 (section 11.2)

Descriptive data

Participants included in the analysis for this objective are described in Table 5.

Outcome and main results

Among the 59 cases that were confirmed to have IS, 47 survived and 12 died. In both groups, vomiting was the most common symptom associated with IS (56/59 94.9). Bloody stool was noted in 68.0% of cases (40/59), and 55.9% (33/59) had abdominal distension. 62.7% of all cases (37/59) had rectal bleeding.

All children diagnosed with IS underwent surgery, except for one child who died before surgery could take place. Among cases who underwent surgery, most (40/57) had their IS manually reduced; in 14 cases, the surgeon performed a resection. In three cases, the surgeon found that the IS had spontaneously reduced. Reduction by contrast enema is not performed in Mali. Ileo-caeco-colic IS was the most common type, accounting for 69.5% (41/59) of all IS.

Table 13. Clinical characteristics of confirmed intussusception cases by outcome

	Survived, n = 47	Died, n = 12

# Female	17 (36.2%)	4 (33.3%)
Median age (range)	6 (1-10)	7.5 (5-9)
Median number of days between symptom onset and admission (range)	3 (0-21)	4.5 (2-10)
Symptoms		
Vomiting	44 (93.6%)	12 (100%)
Abdominal pain	3 (6.4%)	0 (0%)
Abdominal Mass	1 (2.1%)	0 (0%)
Rectal bleeding	36 (76.6%)	1 (8.3%)
Rectal prolapse	1 (2.1%)	0 (0%)
Abdominal Distension	25 (53.2%)	8 (66.7%)
Bloody stool	29 (61.7%)	11 (91.7%)
Rectal Mass	0 (0%)	0 (0%)
Diarrhea	20 (42.6%)	5 (41.7%)
Rectal Distension	0 (0%)	0 (0%)
Dehydration	0 (0%)	0 (0%)
Fever	49 (83.1%)	49 (83.1%)
Rectal pain	0 (0%)	0 (0%)
Lethargy	6 (12.8%)	1 (8.3%)
Gas	3 (6.4%)	1 (8.3%)
Treatment		
Infant died before surgery	0 (0%)	1 (8.3%)
Spontaneous reduction noted before surgery	1 (2.1%)	0 (0%)
Spontaneous reduction noted at surgery	3 (6.4%)	0 (0%)
Surgical reduction required	34 (72.3%)	6 (50%)
Surgical resection performed	9 (19.1%)	5 (41.7%)
Type of IS		
Ileo-caeco-colic	31 (66%)	10 (83.3%)
caeco-colic	1 (2.1%)	0 (0%)
colo-colic	1 (2.1%)	0 (0%)
ileo-colic	5 (10.6%)	0 (0%)
ileoileal	5 (10.6%)	1 (8.3%)
Ileo-caecolic	2 (4.3%)	0 (0%)
Spontaneously reduced	1 (2.1%)	0 (0%)
Unable to determine	1 (2.1%)	1 (8.3%)

Although the mean number of days elapsed between the onset of symptoms and admission to the hospital did not differ significantly between children who survived and those died (3.8 days vs 5 days, p=0.21), no child who died was admitted to the hospital on the same day or day after symptoms began. 38% of children who survived were admitted on the same day or the day after symptoms began. Figure 4 shows the delay between symptom onset and admission for intussusception cases who died and survived. The distribution of survivors includes some children with very large delays to care; the plausibility of these delays is questionable.

Figure 4. Days elapsed between the onset of symptoms and admission to the hospital, by outcome

10.7. **Exploratory objective #5:** To characterize the clinical presentation and severity of MSD illness among enrolled subjects

Participants

All children with MSD will be included in analyses related to this objective. This population is the same as that described in 11.1.4.1 for objective #3.

Descriptive data

For this objective, children will be analyzed in 0-5, 6-11, 12-23 and 24-59 month age groups because we expect that the proportion of cases with rotavirus and the clinical presentation of MSD will differ by age.

Outcome data

The three tables below (Tables 14a – c) show the distribution of scores on elements of the modified Vesikari scale for children enrolled in the MSD by age group. Among all children with MSD, 28.8% (353/1227) experienced vomiting and 38.0% (466/1227) had fever. Nearly all children with MSD (92.5%, 1135/1227) had at least two signs of some dehydration, and an additional 6.3% (77/1227) had at least two signs of severe dehydration. However, IV rehydration and hospitalization as a result of illness were very uncommon. 0.5% received ORS in the health center and 99.7% were prescribed ORS by the health center. Because very few children were admitted to the hospital, because enrollment criteria require that eligible children have a new (onset within 7 days) illness, and because there was no follow-up for duration or intensity of symptoms, the true duration of illness cannot be measured.

Table 14a. Exploratory Objective #5 outcome:
Performance on Components of the Modified Vesikari Scoring Scale, all ages**

	All participants, n = 1227			
Vesikari Score for Parameter	0	1	2	3
Diarrhea				
Maximum # Stools per Day	No diarrhea	1 - 3	5	>=6
N (%)	0 (0%)	509 (41%)	634 (52%)	84 (7%)
Diarrhea Duration (Days)	No diarrhea	1 - 3	5	>=6
N (%)	0 (0%)	1172 (96%)	49 (4%)	6 (0%)
Vomiting				
Maximum # Vomiting Episodes per Day	0	1	2 - 4	>=5
N (%)	874 (71%)	77 (6%)	235 (19%)	41 (3%)
Vomiting Duration	0	1	2	3
N (%)	874 (71%)	134 (11%)	212 (17%)	7 (1%)
Fever	<37.0	37.1-38.4	38.5-38.9	>=39
N (%)	761 (62%)	402 (33%)	61 (5%)	3 (0%)
Dehydration	No or mild dehydration		2 moderate signs	2 major signs
N (%)	15 (1%)		1135 (93%)	77 (6%)
Treatment*	No treatment or ORS	IV Rehydration	Hospitalization	
N (%)	1119 (95%)	1 (1%)	57 (5%)	

* treatment information missing for 50 participants

** The duration of vomiting and diarrhoea includes only days occurring up to the presentation of the child at the health center

Table 14b.
Exploratory Objective #5 outcome:
Performance on Modified Vesikari Scoring Scale,
0 – 5 months & 6 – 11 months

	0 - 5 months, n = 100				6 - 11 months, n = 378			
Vesikari Score for Parameter	0	1	2	3	0	1	2	3
Diarrhea								
Maximum # Stools per Day	No diarrhea	1 - 3	5	>=6	No diarrhea	1 - 3	5	>=6
N (%)	0 (0%)	37 (37%)	56 (56%)	7 (7%)	0	138 (37%)	206 (54%)	34 (9%)
Diarrhea Duration (Days)	No diarrhea	1 - 3	5	>=6	No diarrhea	1 - 3	5	>=6
N (%)	0 (0%)	94 (94%)	4 (4%)	2 (2%)	0	352 (93%)	22 (6%)	4 (1%)
Vomiting								
Maximum # Vomiting Episodes per Day	0	1	2 - 4	>=5	0	1	2 - 4	>=5
N (%)	75 (75%)	8 (8%)	14 (14%)	3 (3%)	236 (62%)	26 (7%)	108 (29%)	8 (2%)
Vomiting Duration	0	1	2	3	0	1	2	3
N (%)	75 (75%)	6 (6%)	18 (18%)	1 (1%)	236 (62%)	43 (11%)	94 (25%)	5 (1%)
Fever	<37.0	37.1-38.4	38.5-38.9	>=39	<37.0	37.1-38.4	38.5-38.9	>=39
N (%)	53 (53%)	43 (43%)	4 (4%)	0 (0%)	228 (60%)	125 (33%)	23 (6%)	2 (1%)
Dehydration	No or mild dehydration		2 moderate signs	2 major signs	No or mild dehydration		2 moderate signs	2 major signs
N (%)	6 (6%)		89 (89%)	5 (5%)	3 (1%)		350 (93%)	25 (7%)
Treatment	No treatment or ORS	IV Rehydration	Hospitalization		No treatment or ORS	IV Rehydration	Hospitalization	
N (%)	89 (94%)	0 (0%)	6 (6%)		329 (87%)	0 (0%)	22 (6%)	

Table 14c. Exploratory Objective #5 outcome:
Performance on Modified Vesikari Scoring Scale, 12 – 23 months and 24 – 59 months

	12 - 23 months, n = 425				24 - 59 months, n = 324			
Vesikari Score for Parameter	0	1	2	3	0	1	2	3
Diarrhea								
Maximum # Stools per Day	No diarrhea	1 – 3	5	>=6	No diarrhea	1 - 3	5	>=6
N (%)	0 (0%)	166 (39%)	228 (54%)	31 (7%)	0 (0%)	168 (52%)	144 (44%)	12 (4%)
Diarrhea Duration (Days)	No diarrhea	1 – 3	5	>=6	No diarrhea	1 - 3	5	>=6
N (%)	0 (0%)	409 (96%)	16 (4%)	0 (0%)	0 (0%)	317 (98%)	7 (2%)	0
Vomiting								
Maximum # Vomiting Episodes per Day	0	1	2 - 4	>=5	0	1	2 – 4	>=5
N (%)	299 (70%)	26 (6%)	77 (18%)	23 (5%)	264 (81%)	17 (5%)	36 (11%)	7 (2%)
Vomiting Duration	0	1	2	3	0	1	2	3
N (%)	299 (70%)	52 (12%)	73 (17%)	1 (0%)	264 (81%)	33 (10%)	27 (8%)	0 (0%)
Fever	<37.0	37.1-38.4	38.5-38.9	>=39	<37.0	37.1-38.4	38.5-38.9	>=39
N (%)	263 (62%)	140 (33%)	22 (5%)	0 (0%)	217 (67%)	94 (29%)	12 (4%)	1 (0%)
Dehydration	No or mild dehydration		2 moderate signs	2 major signs	No or mild dehydration		2 moderate signs	2 major signs
N (%)	3 (1%)		388 (91%)	34 (8%)	3 (1%)		308 (95%)	13 (4%)
Treatment	No treatment or ORS	IV Rehydration	Hospitalization		No treatment or ORS	IV Rehydration	Hospitalization	
N (%)	390 (92%)	1 (0%)	17 (4%)		311 (96%)	0 (0%)	12 (4%)	

The dehydration portion of the modified Vesikari scale shown above comes from the Integrated Management of Childhood Illnesses (IMCI) guidelines for assessing dehydration. IMCI's criteria divide signs of dehydration into three categories: no dehydration, those that suggest some dehydration (slow skin recoil, restlessness/irritability, thirst/eager drinking), and those that suggest severe dehydration (very slow skin recoil, lethargy/unconsciousness, unable to drink/drinking poorly). Sunken eyes can be considered a sign of either some or severe dehydration.

Table 15 shows the prevalence of the IMCI signs of dehydration among the MSD study population. Of these signs, sunken eyes is the most prevalent, having been observed in 99% of enrolled children. The observation that the child appeared thirsty or was drinking eagerly was the second most prevalent sign, being present in 98% of children. The other signs of dehydration were far less common.

Table 15. Prevalence of IMCI signs of moderate and severe dehydration

	All ages, n = 1227	**0 - 5 m, n = 100**	**6 - 11 m, n = 378**	**12 - 23 m, n = 425**	**24 - 59 m, n = 324**
Signs of Some Dehydration	**(n, %)**	**(n, %)**	**(n, %)**	**(n, %)**	**(n, %)**
Skin pinch with slow recoil	468 (38.1)	36 (36)	164 (43.4)	163 (38.4)	105 (32.4)
Restless/Irritable	150 (12.2)	11 (11)	54 (14.3)	64 (15.1)	21 (6.5)
Thirsty, drinks eagerly	1202 (98)	88 (88)	374 (98.9)	419 (98.6)	321 (99.1)
Signs of Severe Dehydration					
Skin pinch with very slow recoil	4 (0.3)	0 (0)	1 (0.3)	3 (0.7)	0 (0)
Lethargic/Unconscious	70 (5.7)	5 (5)	23 (6.1)	30 (7.1)	12 (3.7)
Unable to drink or drinking poorly	3 (0.2)	0 (0)	1 (0.3)	1 (0.2)	1 (0.3)
Signs of Both Some and Severe Dehydration					
Sunken eyes	1215 (99)	99 (99)	373 (98.7)	423 (99.5)	320 (98.8)

Main results for exploratory objective #5

Among the 1227 enrolled MSD cases, the median modified Vesikari score at the time of enrollment was 6. The lowest observed Vesikari was 2, while the highest was 15. The distribution of scores by age group is shown in Table 16.

Table 16. Distribution of Overall Modified Vesikari Scores by Age Group

Age Group	Minimum score	25th percentile	Median	75th percentile	Maximum score
0-5 months (n =100)	2	5	5	7.5	13
6-11 months (n =378)	3	4.75	6	8	15
12-23 months (n =425)	3	4	5	6	15
24-59 months (n =324)	3	5	6	9	14
All ages (n =1227)	2	4	6	8	15

In other studies, children with modified Vesikari scores less than 7 have been classified as 'mild' severity, while those with scores greater than or equal to 7 but less than 11 have been classified as "moderate" severity. Children with scores greater than or equal to 11 but less than 15 were classified as "severe," and those scored 15 or greater were "very severe." Using this classification system, 66.4% of the children enrolled had mild illness, while 33.6% had moderate-to-very severe illness.

Table 17. Distribution of Modified Vesikari Score Severity Categories by Age Group

Age Group	Mild (Vesikari score <7)	Moderate (Vesikari score 7-10)	Severe (Vesikari score 11-14)	Very Severe (Vesikari score 15-20)
0-5 months (n =100)	70 (73.7%)	17 (17.9%)	8 (8.4%)	0 (0%)
6-11 months (n =378)	199 (56.7%)	115 (32.8%)	37 (10.5%)	0 (0%)
12-23 months (n =425)	258 (63.2%)	125 (30.6%)	24 (5.9%)	1 (0.2%)
24-59 months (n =324)	254 (78.6%)	59 (18.3%)	9 (2.8%)	1 (0.3%)

All ages (n =1227)	781 (66.4%)	316 (26.8%)	78 (6.6%)	2 (0.2%)

10.8. Other analyses

Not applicable at this time.

10.9. Adverse events/adverse reactions

No vaccine or drug was administered as part of this study. However, when study investigators became aware of a child who received RotaTeq and experienced an event that met the definition of a severe adverse event (SAE), that event was reported to Merck Global Safety, whether or not the child was enrolled in the present study. An SAE was defined as "an adverse event which is fatal or life threatening, results in persistent or significant disability/incapacity, requires inpatient hospitalization, prolongation of existing inpatient hospitalization, or is a congenital anomaly/birth defect, cancer, the result of an overdose or is another important medical event." Per our Safety Management Plan, we did not report suspected intussusception cases to Merck Global Safety until after adjudication took place, except for in the case of deaths or other serious outcomes.

During the study, including the run-in period, 75 children who had been vaccinated with RotaTeq experienced SAEs that were reported to Merck Global Safety (MGS). 7 of these children were discovered in the course of activities related to the MSD portion of the study—all 7 were deaths, related to pneumonia (1), burns (2), gastroenteritis (2), malnutrition (1), and malaria (1). One of the children who died from a burn was not enrolled in the MSD study, but as a control in the VIDA study, which enrolls healthy children as controls for children with diarrhea—this child had been vaccinated with RotaTeq and died during his time under follow-up.

The remaining 68 children were enrolled in the IS study. 65 of these children suffered intussusceptions, confirmed by adjudication; subsequent to their intussusceptions, two of these children experienced wound dehiscence, one experienced intestinal perforation, one experienced cardio-respiratory arrest, gastrointestinal obstruction, and pneumonia, and one experiences an anaesthetic complication followed by loss of consciousness, fever and convulsions. 13 of the children with confirmed intussusceptions died. The three remaining children enrolled in the IS study who did not have a confirmed intussusception died prior to adjudication. One of these children had a bowel obstruction and was severely malnourished, one had a bowel obstruction complicated by a wound dehiscence, and one had a volvulus.

Table 18. Serious adverse events reported from MSD and IS studies

Type of Case	Number reported	Type of Event
Child enrolled in diarrhea study	6	• 6 deaths associated with: ○ Burns ○ Gastroenteritis (2) ○ Pneumonia ○ Severe acute malnutrition ○ Malaria
Child enrolled in IS study	75	• 16 deaths associated with: ○ Intestinal obstruction and malnutrition ○ Intestinal obstruction and wound dehiscence ○ Volvulus ○ Intussusception (13) • 52 non-fatal intussusceptions
Child identified over the course of other study activities	1	• 1 death associated with: ○ Burn PPD

During the study, investigators did not identify any non-severe adverse events. Moderate-to-severe diarrhea following at least one dose of RotaTeq, which is not an adverse event per our study protocol, occurred in 1123 out of 1380 AGE cases enrolled during the run-in or main study period .

10.10. Protocol deviations

During the study there have been several protocol deviations. Table 19 below shows protocol deviations identified and submitted to the IRB. Except for the first deviation listed, all deviations originated from discrepancies between our protocol and a study protocol that was being carried out simultaneously in the same population. It was the investigators' initial intention that these protocols not differ, so amendments will be made to the protocols to ensure that study procedures are uniform.

Table 19. Protocol deviations identified since start of study

Deviation description	Type of deviation	Result of deviation	Impact of deviation	Was this deviation reported?	Actions to prevent from reoccurrence
Vaccination status was not removed from the IS packages for the Adjudication Committee to review and a member became unblinded.	Other	AC member became unblinded as to vaccination status	IS package will be reviewed by another AC member	Yes, to UMB IRB and Mali IRB	All unredacted vaccination information was redacted; we asked an alternative committee member to provide adjudication in place of the member who saw the vaccination information. Improved QA processes were implemented.
According to the Merck protocol, the team must include all eligible cases aged 0-59 months during the study period. However, 7 cases were not enrolled because the clinical team capped enrollment at 8-9 cases per age group per fortnight in accordance with a companion (VIDA) protocol.	Eligibility/Enrollment	Missed enrollment opportunities	Study integrity has not been impacted.	Yes, to UMB IRB and Mali IRB	The team was summoned to inform them of the differences between the protocols.
Per Merck protocol, cases can be re-enrolled after seven diarrhea-free days, but cases were not re-enrolled until 60 days elapsed in accordance with a companion (VIDA) protocol	Eligibility/Enrollment	Missed enrollment opportunities	Study integrity has not been impacted.	Yes, to UMB IRB and Mali IRB	Protocol will be updated to harmonize with the VIDA protocol.

Per Merck protocol, eligible cases must provide a stool sample within 24 hours of enrollment, but cases were followed only 12 hours for a stool sample in accordance with a companion (VIDA) protocol .	Eligibility/Enrollment	Missed enrollment opportunities	Study integrity has not been impacted.	Yes, to UMB IRB and Mali IRB	Protocol will be updated to to harmonize with the VIDA protocol
Cases who did not provide a rectal swab prior to antibiotic administration were erroneously excluded from the study	Eligibility/Enrollment	Missed enrollment opportunities	Study integrity has not been impacted.	Yes, to UMB IRB and Mali IRB	Study team was informed that these children should be enrolled in the study

11. DISCUSSION

11.1. KEY RESULTS

After a three month run-in period, official study enrolment began on August 1, 2015 for both the MSD and intussusception studies. During MSD surveillance, 1227 cases were enrolled and six rounds of HUCS surveillance were carried out, and we observed an annual rotavirus-associated MSD incidence rate of 5.88 cases per 100 child-years among children 6-23 months of age who were fully vaccinated with RotaTeq (95% CI: 2.97 – 8.80). During intussusception surveillance, 83 suspected IS cases were enrolled and adjudicated; of these, 59 were confirmed as IS. Based on the findings of the first Bamako-wide vaccine coverage survey, we estimated that the annual IS rate among children one month after a dose of RotaTeq is 5.83 per 100,000 child-years. The annual incidence of IS among all Bamako infants regardless of vaccination status is 30.07 per 100,000 child-years. During this study, no new safety signals were detected. Because the study was terminated early, and because the new formulation of RotaTeq was never released, we are not able to perform the planned pre/post analyses.

11.2. LIMITATIONS

Because gastroenteritis cases were enrolled from sentinel health centres, children enrolled in our study may differ from gastroenteritis cases in the community who did not present to health centres. Although we assumed that intussusception cases would present to a

health centre due to the severity of symptoms, it is possible that some children died or experienced a spontaneous reduction before seeking care.

11.3. INTERPRETATIONS

Because this study was terminated before its primary objectives could be assessed, there are no interpretations.

11.4. GENERALIZABILITY

The results of this study may be generalizable to other African countries where RotaTeq is in use.

12. OTHER INFORMATION

13. CONCLUSIONS

Because this study was terminated before its primary objectives could be assessed, there are no conclusions.

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ANNEX 1 LIST OF STAND-ALONE DOCUMENTS

Number	Document reference number	Date	Title
1	EP08011.027	December 23, 2014	A Study of the Effectiveness and Safety of a New Formulation of RotaTeq™ in Routine Use in a Developing World Setting
2		September 10, 2015	Data Management Plan for the Teleforms System
3		June 25, 2015	VIDA Rotavirus Vaccine Impact on Diarrhea in Africa (VIDA) Data Management Plan
4		September 17, 2015	Statistical Analysis Plan
5		July 27, 2015	Standard Operating Procedure for the

Number	Document reference number	Date	Title
			Conduct of Vaccine Coverage Surveys for a Post-Licensure Study of RotaTeq™ in Mali

ANNEX 2 VACCINE COVERAGE SURVEY

The second primary objective requires the vaccination rates for children less than a year old residing in Bamako. To estimate this rate, we conducted two vaccine coverage surveys among children who were 9 to 21 months of age, one in each year of the study. Because we could not enumerate and sample all children living in Bamako, we sampled children in two stages. First, we selected 50 clusters from 1047 population clusters with probability proportional to the cluster's size (these are the "primary sampling units"), then we sampled 10 children from each cluster by randomly selecting cells from a grid superimposed upon the primary sampling unit and enrolling the first eligible child discovered in the cell.

During the two vaccine coverage surveys conducted, 1157 children were contacted and evaluated for eligibility. There were 122 children who fell outside of the survey age limits and were excluded from the survey. Of the 1035 children remaining, 31 were excluded because they had not resided in Bamako for at least six months and were thus considered "non-residents." 3 of the 1004 children who remained were excluded from analysis because their vaccination records were inaccessible. Three additional children had a vaccination cards that did not definitively determine RotaTeq status, so these children were omitted and a total of 998 children were included. No caregiver refused to give consent for her child's enrollment.

A cluster sampling method has been employed to minimize bias in selection of children to be surveyed given the absence of a household listing for Bamako. Because we sampled primary sampling units in proportion to population, it is not necessary to weight the results of the coverage survey. Vaccine coverage in Bamako can therefore be estimated by:

$$\hat{p} = \frac{\#\ Fully\ vaccinated}{\#\ Sampled}$$

The standard error of RotaTeq coverage can be estimated by:

$$SE(t) = \sqrt{\frac{\hat{p}\ \times\ (1 - \hat{p})}{n}}$$

Among children participating in the two combined surveys, we found that 56 (6%) had received no doses of RotaTeq, 53 (5%) had received only one dose, 61 (6%) had received only two doses, and 828 (83%) had received all three doses. Of those who received three doses, 792 (95%) received all three doses before 33 weeks of age, in compliance with RotaTeq's dosing schedule. Two children was known to have received three doses of RotaTeq, but did not have dates associated with the doses and so could not be classified as either fully vaccinated or not fully vaccinated.

The complete coverage of RotaTeq (receipt of three doses before 33 weeks of age, per the EPI schedule) in this population was estimated to be 792/996, or 80%. The standard error was 1.8%, and the two-sided 95% confidence interval (CI) for complete coverage was (76%, 83%). Coverage with exactly one dose was 6% (95% CI: 4% - 7%). Coverage with exactly two doses was 6% (95% CI: 5% - 8%). Coverage with exactly three doses (whether or not they were administered before 33 weeks of age) was 83% (95% CI: 80% - 86%).

We estimated that 3.6% of the population of Bamako during this time period were children less than one year of age. According to the 2009 census, the total population of Bamako was 1,810,366. INSTAT and the World Bank have estimated that the annual growth rate for the city of Bamako is 5.4%, so the estimated number of people living in Bamako at the beginning of 2016 was 2,616,078, which we treat as the population midpoint for the IS portion of this study. From this, we estimated that the number of infants living in Bamako in 2016, if infants comprised 3.6% of the population, was 94,179, which is somewhat higher than the birth cohort estimated in the protocol (89,583). The estimate that infants make up 3.6% of the population comes from the 2013 DHS survey. The estimated number of infants fully vaccinated with RotaTeq, therefore, is 94,179 times the proportion of children with full RotaTeq coverage. In 2016, this means that the fully vaccinated population was estimated as 74,889 (95% CI: 71,6561 to 78,127). The number of children vaccinated with at exactly 1, 2 and 3 doses is estimated using the same method. The number of children with exactly one dose of RotaTeq is estimated to be 5,285 (95% CI: 3,539 to 7,030), with exactly two doses is 5,756 (95% CI: 4,327 to 7,185), and with exactly three doses is 78,136 (95% CI: 75,128 to 81,145).

Table 19 shows characteristics of children enrolled in the vaccine coverage survey for all of Bamako and by commune. 463 of the 998 analyzable respondents 46.0%) were female (two had no sex recorded) and the mean age of respondents was 14.9 months (range: 9-20 months). Since only 6 (0.6%) of eligible subjects did not provide immunization coverage data, a comparison of the demographic features of these children to those who provided coverage data is not shown.

Table 19. Characteristics of Children Surveyed for Vaccine Coverage by Commune

	All Bamako	Commune 1	Commune 2	Commune 3	Commune 4	Commune 5	Commune 6
# Children	998	242	110	50	149	210	237
Mean age (months)	14.9	15.1	14.3	14.8	15.0	15.0	14.9
Female sex (%)	463 (46%)	107 (44%)	49 (45%)	28 (56%)	65 (44%)	99 (47%)	115 (49%)
Maternal Education							
No Formal Schooling	441 (44%)	112 (46%)	47 (43%)	16 (32%)	56 (38%)	92 (44%)	118 (50%)
Primary schooling	333 (33%)	91 (38%)	41 (37%)	20 (40%)	49 (33%)	64 (30%)	68 (29%)
Secondary schooling	162 (16%)	23 (10%)	19 (17%)	13 (26%)	31 (21%)	40 (19%)	36 (15%)
Post-secondary schooling	54 (5%)	14 (6%)	2 (2%)	1 (2%)	11 (7%)	13 (6%)	13 (5%)
Immunization Data source							
Immunization card available of first visit (%)	966 (97%)	225 (93%)	106 (96%)	47 (94%)	148 (99%)	208 (99%)	232 (98%)
Immunization card sole data source (%)	32 (100%)	17 (100%)	4 (100%)	3 (100%)	1 (100%)	2 (100%)	5 (100%)
RotaTeq Doses received (%)							
0 doses	56 (6%)	8 (3%)	2 (2%)	3 (6%)	2 (1%)	10 (5%)	31 (13%)
1 dose	53 (5%)	15 (6%)	3 (3%)	2 (4%)	7 (5%)	14 (7%)	12 (5%)
2 doses	61 (6%)	7 (3%)	3 (3%)	1 (2%)	9 (6%)	21 (10%)	20 (8%)
3 doses	828 (83%)	212 (88%)	102 (93%)	44 (88%)	131 (88%)	165 (79%)	174 (73%)

Fully vaccinated by 33 weeks of age	792 (80%)	207 (86%)	97 (88%)	44 (88%)	130 (87%)	150 (71%)	164 (69%)

ANNEX 3 HUCS ROUNDS

Exploratory objective #2, estimation of RotaTeq coverage in the MSD catchment area, is a population statistic estimated from HUCS surveys, which are carried out in the DSS at least twice per year. Additionally, the HUCS surveys will be used with data from the healthcare centers to estimate the proportion of rotavirus MSD cases that seek healthcare, which are required for both the first primary objective and the third exploratory objective. Six HUCS surveys and DSS rounds were completed or began during the first full year of enrollment (Table 20).

Table 20. HUCS and DSS rounds during first year of MSD enrollment

	HUCS		DSS	
	Start Date	**End Date**	**Start Date**	**End Date**
First Round	15-Jun-15	15-Sep-15	8-May-15	10-Sep-15
Second Round	2-Nov-15	26-Feb-16	2-Nov-15	23-Feb-16
Third Round	2-May-16	28-Sep-16	2-May-16	28-Sep-16
Fourth Round	13-Oct-16	23-Jan-17	13-Oct-16	19-Jan-17
Fifth Round	10-Apr-17	21-Aug-17	10-Apr-17	15-Aug-17
Sixth Round	7-Nov-17	12-Feb-18	7-Nov-17	1-Mar-18

For each HUCS round, an age-stratified (0-11 mo. [n=550], 12-23 mo. [n=500],] and 24-59 mo. [n=500]) random sample was to be drawn from the DSS database. Over the six rounds, 11895 children were selected for enrollment in the study; 1817 of these children were not enrolled. Table 21 shows that 1568 (86%) of these children were ineligible. The remainder (249/1817 (14%)) were not enrolled because the child was considered a non-responder (the primary caretaker was not available or could not be located after 3 attempts) or refusal. The analysis will adjust for non-response and refusal in the weights that are assigned to each child for whom information is obtained using the DSS sample as a whole. It is notable that caregiver refusal accounted for less than one percent of non-enrollment.

Table 21. Reasons for non-enrollment during first three HUCS rounds

Reason not enrolled	First round	Second round	Third round	Fourth round	Fifth round	Sixth round
Child ineligible (replaced)						
Child died	9 (3.4%)	6 (2.3%)	12 (2.8%)	26 (8.9%)	14 (4.8%)	6 (2.2%)
Child traveling	130 (48.7%)	101 (38.3%)	198 (46.2%)	122 (41.9%)	130 (44.7%)	123 (44.7%)
Moved away	89 (33.3%)	114 (43.2%)	153 (35.7%)	114 (39.2%)	111 (38.1%)	110 (40%)
Non-responders (kept in sample)						
Primary caretaker not available	30 (11.2%)	32 (12.1%)	56 (13.1%)	24 (8.2%)	33 (11.3%)	32 (11.6%)
Unable to locate child	9 (3.4%)	10 (3.8%)	6 (1.4%)	4 (1.4%)	1 (0.3%)	4 (1.5%)
Refused (kept in sample)	0 (0%)	1 (0.4%)	4 (0.9%)	1 (0.3%)	2 (0.7%)	0 (0%)

Consent for enrollment was granted for 10078 (98%) of the 10327 eligible children selected for enrollment into the HUCS survey. For the purposes of the first year analysis, all six HUCS surveys were combined to estimate two statistics. First, we estimated the prevalence of a full course of RotaTeq among various age groups in the DSS (0-5 months, 6-11 months, 12-23 months, and 24-59 months, as well as 6-23 months of age, which reflect the age group under study for primary objective #1). Second, we estimated the proportion of moderate-to-severe rotavirus-associated diarrhea that resulted in healthcare seeking among the age groups mentioned above.

For the purposes of estimating the proportion of moderate-to-severe rotavirus-associated diarrhea that results in healthcare seeking at the VIDA sentinel health centers (r), the mean mid-point DSS population was taken to be the mean of the six DSS rounds conducted over the course of the study. Sampling fractions were specific to the DSS round and age group.

The sampling weight, which is equal to the number of children in the DSS in an age group divided by the number of children sampled from that age group, is shown for each round in Table 22. This is calculated from the mid-point population for the DSS round associated with the HUCS round. Sampling fractions ranged from 2.2 (female 6 – 11 month olds during the third round) to 69 (female 0 – 5 month olds during the second round).

Estimates of r are shown in Table 23. r is estimated as 1-$S(t)$, where $S(t)$ is the survival function at time t as estimated by the Kaplan-Meier method.

Table 22. HUCS enrollment and sampling weight during first three rounds, by age

Age Group	Gender	First Round			Second Round			Third Round		
		# Approached	# Enrolled	Sampling weight	# Approached	# Enrolled	Sampling weight	# Approached	# Enrolled	Sampling weight
0-5 months	Male	51	50	12.1	30	30	18.3	74	69	9. 7
0-5 months	Female	41	41	14.9	30	29	18.0	66	65	10.3
6-11 months	Male	214	213	3.5	260	253	2.7	376	363	2.2
6-11 months	Female	206	202	3.6	243	234	2.7	382	369	2.2
12-23 months	Male	220	212	7.0	272	264	7.2	435	428	3.6
12-23 months	Female	215	204	7.2	297	287	6.8	368	360	4.2
24-59 months	Male	198	193	24.8	282	277	15.9	447	437	10.4
24-59 months	Female	228	219	21.0	276	273	16.4	373	364	12.3

Age Group	Gender	Fourth Round			Fifth Round			Sixth Round		
		# Approached	# Enrolled	Sampling weight	# Approached	# Enrolled	Sampling weight	# Approached	# Enrolled	Sampling weight
0-5 months	Male	77	77	8.2	14	13	45.3	90	89	7.4
0-5 months	Female	74	72	8.4	9	9	69.0	66	66	9.8
6-11 months	Male	170	164	4.1	269	266	2.8	229	220	3.8
6-11 months	Female	173	171	4.1	259	254	2.8	224	220	3.6
12-23 months	Male	234	229	6. 3	263	257	5.8	294	291	5.5
12-23 months	Female	232	226	6.3	256	252	5.8	261	255	6.2
24-59 months	Male	232	226	19.7	248	240	17.9	311	304	14.8
24-59 months	Female	219	217	20.3	249	240	17.6	290	284	15.8

Table 23. r values calculated for HUCS surveys

Age group	1 - S(t)	Upper 95% CI	Lower 95% CI
0 - 5 months	0.14	0.08	0.19
6 - 11 months	0.27	0.12	0.39
12 - 23 months	0.22	0.14	0.29
24 - 59 months	0.27	0.21	0.33
6 - 23 months	0.23	0.16	0.29

To estimate RotaTeq coverage, HUCS interviewers ask to review the enrolled child's vaccination card to ascertain vaccination status. If the interviewer is unable to review the card, or if the card has been lost, the interviewer will review the DSS vaccination records and if the data cannot be obtained they go to the health center where the child was vaccinated to review records. Nonetheless, it was common that a child's vaccination status could not be assessed. Vaccination record recovery varied from 98% among 0-5 month olds in the second round to 66% among 24-59 month olds in the sixth round. In general, older children were less likely to have vaccination information, and therefore the sampling weights for these age groups increased since more children in the DSS were represented by a single surveyed child. We assumed that vaccination information within age groups is missing completely at random.

Table 24. HUCS enrollees with vaccination information & sampling fraction

	First Round			Second Round			Third Round		
Age Group	# enrolled	# with vaccination information	% without vaccination information	# enrolled	# with vaccination information	% without vaccination information	# enrolled	# with vaccination information	% without vaccination information
0-5 mo.	91	82	10%	59	58	2%	134	126	6%
6-11 mo.	415	354	15%	487	472	3%	732	685	6%
12-23 mo.	416	299	28%	551	490	11%	788	680	14%
24-59 mo.	412	239	42%	550	406	26%	801	582	27%
6 - 23 mo.	831	653	21%	1038	962	7%	1520	1365	10%
	Fourth Round			**Fifth Round**			**Sixth Round**		
Age Group	# enrolled	# with vaccination information	% without vaccination information	# enrolled	# with vaccination information	% without vaccination information	# enrolled	# with vaccination information	% without vaccination information
0-5 mo.	149	137	8%	22	21	5%	155	144	7%
6-11 mo.	335	312	7%	520	479	8%	440	387	12%
12-23 mo.	455	405	11%	509	443	13%	546	420	23%
24-59 mo.	443	326	26%	480	323	33%	588	332	44%
6 - 23 mo.	790	717	9%	1029	922	10%	986	807	18%

ANNEX 4 STATISTICAL METHODS

We calculate the annual incidence for rotavirus-associated MSD *(q)*, as shown below:

$$q = \frac{y}{n}$$

where *n* is the annual midpoint number of children residing in the demographic surveillance system area in the age group of interest who are fully vaccinated, as estimated by the midpoint number of children in the age group times the proportion of children in that age group who are fully vaccinated. *y* represents the number of children who had rotavirus-associated moderate-to-severe diarrhea during the first year of the study, calculated by:

$$y = \frac{msp}{r}$$

where *m* is the number of children in an age group with moderate-to-severe diarrhea during the first year of the study, *p* is the proportion of enrolled children in that age group who were fully vaccinated with RotaTeq and fell ill with rotavirus at least 14 days after their final RotaTeq dose, and *r* is the proportion of children with moderate-to-severe diarrhea who are taken to a sentinel health care center. Since we have observed one year's worth of cases, the number of children observed is equal to the number of child-years observed.

Estimation of *r* is described and shown in Appendix 3.

Table 25. Rotavirus-associated MSD incidence rate among fully vaccinated children, 6-23 mo

Age Group	# eligible MSD cases annually (*m*)	Pr(Rotavirus MSD | fully vaccinated > 14 days) (*p*)	*r*	y_{mp}	RotaTeq coverage in DSS	DSS mid-point Population	Estimated # fully vaccinated children (*n*)	Estimated Incidence per 100 child-years
6 - 11 months	183.3	0.143	0.269	97.7	0.868	1482.3	1286.7	7.60 (1.68 - 13.51)
12 - 23 months	211.3	0.139	0.220	133.4	0.855	3145.2	2689.1	5.00 (1.82 - 8.10)
6 - 23 months	394.7	0.141	0.229	231.2	0.860	4627.5	3979.7	5.81 (2.93 - 8.70)

ANNEX 5 STUDY PROTOCOL

ANNEX 6 ADDITIONAL INFORMATION