Vaccine Impact on Diarrhea in Africa (VIDA)

Synopsis

Sites:

Overall Study Coordinating Center

Center for Vaccine Development (CVD)
University of Maryland School of Medicine, Baltimore, Maryland 21201, USA

Participating Field Sites

1. Centre pour le Développement des Vaccins du Mali (CVD-Mali), Bamako, MALI
2. Medical Research Council (MRC), Basse, THE GAMBIA
3. CDC/Kenya Medical Research Institute (KEMRI) Research Station, Siaya County, KENYA

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Umesh Parashar, MBBS (Epidemiologist)
Ciara O'Reilly, PhD (Epidemiologist)
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Study design: Observational

1. Health care utilization and coverage survey (HUCS) semiannually – to be conducted as part of the Demographic Surveillance System at each site with the data linked
2. Case-control study of the etiology, and adverse clinical consequences of moderate-to-severe diarrhea (MSD); data from the case-control study will also be used to measure rotavirus vaccine impact and effectiveness

Subjects: (Total 31,500)

Case control study: up to 1,000 cases with MSD and 2,500 control children per year per site (total 10,500 per site, and 31,500 overall)

Note: At each site, participants will be recruited from a censused population with an ongoing Demographic Surveillance System (DSS) which conducts rounds at least twice per year to enumerate and characterize the population in order to measure disease incidence. The DSS will not be conducted under the current protocol, although data will be shared. Similarly, the HUCS is conducted under the DSS, not the current protocol, although data will be shared.

Duration:

Overall clinical activity: 39 months per site

1. HUCS: semiannually for 36 months; ~15 minutes per interview
2. Case-control study: enrollment for 36 months; each child participates for up to ~90 days

Study objectives:

This project represents an extension of the Global Enteric Multicenter Study (GEMS, UMB IRB# HP 40030), utilizing similar methodologies as in GEMS to meet the following objectives:

1. To assess the impact of rotavirus vaccine introduction on the:
   a. Etiology of MSD, by comparing the number and proportion of MSD cases with specific pathogens before rotavirus vaccine introduction (i.e., during GEMS) and in the study period, as well as comparing adjusted pathogen-specific incidence and attributable fractions;
   b. Adverse clinical consequences of MSD, measured as persistent diarrhea, linear growth attainment, and mortality during a 2-3 month follow-up period after study enrollment;
   c. Overall incidence of MSD; and
   d. As an exploratory objective, diarrhea- associated under-5 mortality in the DSS population measured using verbal autopsy.
2. To provide a well-characterized library of stool samples for analysis by TaqMan®, a highly sensitive, quantitative molecular assay, to more precisely define the pathogen-specific diarrheal disease burden.
3. To determine the effectiveness of a full course of rotavirus vaccine using a case-control study design with two separate control populations: rotavirus test-negative cases with MSD, and matched community controls. Additional information (exploratory) that can be gleaned from the case-control study includes:
   a. Effectiveness of a partial course of rotavirus vaccine;
   b. Duration of protection;
   c. Presence of protection in children too young to receive vaccine (herd immunity); and
   d. Potential risk factors for vaccine failure, including nutritional status and concomitant infection with enteropathogens, such as *Giardia*, soil helminths, and *H. pylori*, that may modulate the immune system or the expression of diarrheal symptoms.
4. To test, in an exploratory fashion, whether genetic mutations in histo-blood group antigens *FUT2* (secretor) and *FUT3* (Lewis) are associated with an increased risk of selected enteropathogens, including moderate-to-severe rotavirus diarrhea, among vaccinated children.

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1. List of abbreviations

CDC	Centers for Disease Control and Prevention (Atlanta, Georgia, USA)
CFA	Colonization Factor Antigen of Enterotoxigenic *Escherichia coli*
CI	Confidence Interval
CIN	Cefsulodin Irgasan Novobiocin agar
CRF	Case Report Form
CVD	Center for Vaccine Development (Baltimore, Maryland, USA)
CVD-Chile	Centro para Vacunas en Desarrollo (Santiago, Chile)
CVD-Mali	Centre pour le Développement des Vaccins du Mali (Bamako, Mali)
DCC	Data Coordinating Center
DSS	Demographic Surveillance System
EAEC	Enteroaggregative *E. coli*
EHEC	Enterohemorrhagic *E. coli*
EIA	Enzyme Immunoassay
EIEC	Enteroinvasive *E. coli*
EPEC	Enteropathogenic *E. coli*
ETEC	Enterotoxigenic *E. coli*
EPI	Expanded Programme on Immunization (site of routine infant immunizations)
GCP	Good Clinical Practice
HAZ	Height for Age Z Score
HUCS	Health Care Utilization and Coverage Survey
IRB	Institutional Review Board
KEMRI	Kenya Medical Research Institute
LT	Heat Labile Toxin of ETEC
MDM	Molecular Diagnostics and Microbiology Section of the CVD
MRC	Medical Research Council (Basse, The Gambia)
MSD	Moderate-to-severe diarrhea
MUAC	Mid-upper arm circumference
PCR	Polymerase Chain Reaction
RNA	Ribonucleic Acid
RT-PCR	Reverse Transcriptase-Polymerase Chain Reaction
PI	Principal Investigator
SD	Standard Deviation
SOP	Standard Operating Procedure
S-S	*Salmonella-Shigella* Agar
ST	Heat stable toxin of ETEC
TCBS	Thiosulfate citrate bile salts sucrose agar
TSI	Triple sugar iron agar
UMB	University of Maryland, Baltimore
QA	Quality Assurance
QC	Quality Control
VA	Verbal autopsy
WHO	World Health Organization
XLD	Xylose-Lysine-Desoxycholate Agar

2. Executive summary

Despite considerable progress in reducing diarrhea-related mortality over the past two decades, diarrheal diseases remain the second leading cause of post-neonatal death during the first 5 years of life in developing countries. As further declines are made possible by expanding interventions that target the principal causes of severe disease, the availability of accurate, up-to-date assessments at the country-level are essential to guide strategic planning and resource allocation. This is especially true for sub-Saharan Africa and South Asia, where 82% of the under-five deaths are now concentrated, and where current, systematically-collected information on the burden and major causes of child disease and death has been lacking. To address these knowledge gaps, the Global Enteric Multicenter Study (GEMS) was conducted in 7 developing country sites to elucidate the incidence, etiology, and adverse outcomes of moderate-to-severe diarrhea (MSD) among children 0-59 months of age. GEMS demonstrated the large burden of MSD and its significant association with linear growth faltering and death in the selected low resource settings. Unequivocally, rotavirus was the most common etiology of MSD during the first 2 years of life, while other pathogens, e.g., *Cryptosporidium* and heat-stable toxin-producing enterotoxigenic *Escherichia coli* (ST-ETEC), contributed significantly to poor growth and mortality.

During the next few years, rotavirus vaccines are expected to be introduced into routine infant immunization programs across low-income countries, and marked reductions in child deaths and hospitalizations from rotavirus diarrhea are anticipated. However, the impact of vaccine introduction on the epidemiology of diarrheal diseases will likely extend beyond changes in rotavirus-associated morbidity and mortality alone; shifts in the predominant pathogens and adverse outcomes associated with MSD are also expected. Continued progress in the control of diarrheal diseases thus will require a new fund of knowledge to develop and prioritize strategies that are relevant and appropriate to the causes and consequences of diarrheal diseases in the future. The rapidly emerging field of molecular diagnostics can now be applied to define more precisely the pathogen-specific disease burden; these techniques offer advantages over conventional microbiology, including the application of a standard methodology for all pathogens, a mechanism for quantifying microbial load, thereby helping to distinguish infection from colonization, and an increased ability to identify bacteria in the face of recent antibiotic use.

This proposal aims to utilize the established GEMS infrastructure and methodology to conduct a 36-month case-control study of MSD in three prototypic low income countries in sub-Saharan Africa (Mali, The Gambia, and Kenya) to provide a detailed assessment of the changes in incidence, etiology, and adverse clinical outcomes of MSD that emerge once rotavirus vaccine has been introduced. The objectives of this study are: 1) to assess the impact of rotavirus vaccine introduction on the incidence, etiology, and adverse clinical consequences of MSD; 2) to provide a well-characterized library of stool samples for analysis using highly sensitive, quantitative molecular assays; and 3) to determine the effectiveness of rotavirus vaccine using a case-control study design, including the duration of protection, and potential risk factors for vaccine failure. One such risk factor for vaccine failure of interest is genetic mutations in histo-blood group antigens.

3. Background and rationale

Nearly one in 10 child deaths that occur during the first 5 years of life are attributed to diarrheal diseases.[1] Although there is evidence to suggest that diarrhea-related deaths among children are decreasing, the number remains unacceptably high, with an estimated 600,000 fatalities worldwide annually.[1] As further declines are made possible by expanding interventions that target the most severe causes of disease, the availability of accurate, up-to-date assessments at the country level becomes even more important to guide strategic planning and resource allocation. This is especially true for sub-Saharan Africa, where 50% of the estimated annual 6.5 million under-five deaths are now concentrated and where current, systematically-collected information on the burden and major causes of child disease and death has been lacking.

The Global Enteric Multicenter Study (GEMS)[2] was conducted to address these knowledge gaps by assessing the incidence, etiology, and adverse clinical outcomes of moderate-to-severe diarrhea (MSD) among children aged 0-59 months residing in censused populations at 7 sites in sub-Saharan Africa and South Asia.[3] We estimated the proportion of MSD that could be prevented with targeted interventions using adjusted population attributable fractions (AF) for each pathogen.[3,4] GEMS demonstrated the large burden of MSD and its significant association with linear growth faltering and death in the selected low resource settings.[3] Four pathogens were found to comprise the majority of attributable cases of MSD across sites: rotavirus, *Cryptosporidium*, enterotoxigenic *Escherichia coli* (ETEC) producing heat stable toxin (ST-ETEC) either alone or with heat labile toxin (LT-ETEC), and *Shigella*. Unequivocally, rotavirus was the most common pathogen during the first 2 years of life. Nearly 25% of MSD is attributable to rotavirus during infancy (**Table 1**), demonstrating the highest AF of any pathogen at every site during infancy.

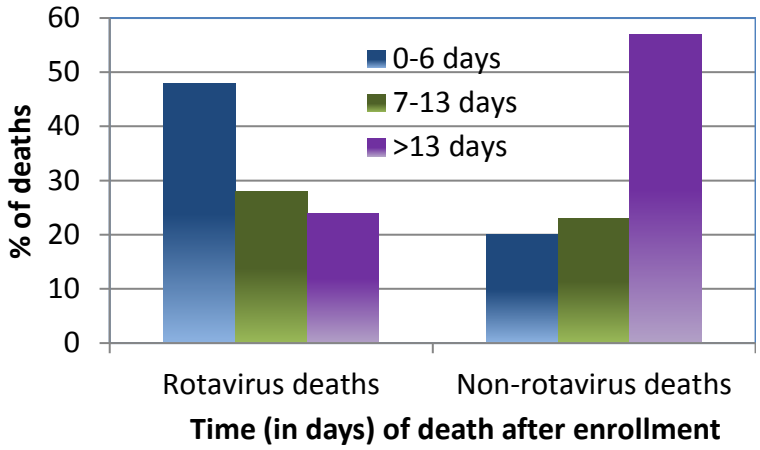
Table 1. Adjusted attributable fraction (expressed as weighted percent of total episodes) and weighted annual incidence (per 100 child-years) of rotavirus-associated moderate-to-severe diarrhea, by age stratum and site

Age stratum	Basse, The Gambia	Bamako, Mali	Manhiça, Mozambique	Siaya Co., Kenya	Kolkata, India	Mirzapur, Bangladesh	Karachi, Pakistan
Weighted, adjusted attributable fraction (95% CI):							
0-11 mo.	23·5 (18·8-28·2)	21·7 (18·3-25·2)	27·8 (21·0-34·6)	19·7 (16·4-23·1)	27·0 (23·3-30·6)	16·3 (12·8-19·8)	22·6 (18·9-26·3)
12-23 mo.	17·0 (13·4-20·6)	11·9 (9·0-14·7)	-	13·3 (9·7-17·0)	25·4 (21·5-29·2)	18·3 (14·5-22·1)	9·8 (6·4-13·3)
24-59 mo.	12·1 (6·6-17·6)	3·0 (0-21·9)	-	3·5 (1·3-5·6)	14·5 (10·2-18·7)	-	-
Weighted annual incidence of RV MSD (per 100 child-years):							
0-11 mo.	3·2 (1·7-4·6)	8·4 (3·5-13·3)	3·5 (1·5-5·4)	10·1 (5·4-14·8)	25·4 (14·7-36·2)	2·1 (1·0-3·2)	5·5 (2·6-8·5)
12-23 mo.	3·3 (1·3-5·2)	4·1 (1·0-7·1)	-	3·0 (1·6-4·3)	12·0 (6·8-17·2)	-	-
24-59 mo.	0·4 (0·1-0·6)	0·4 (0-3·2)	-	0·3 (0·1-0·4)	3·5 (0-7·1)	-	-

While its AF generally diminished with age, rotavirus had the largest AF of any pathogen among toddlers at four sites, and at The Gambia and India sites even in the eldest stratum. In a pooled analysis across sites, MSD cases had significantly more linear growth faltering (decrease in height-for-age z [HAZ] scores) than controls at the follow-up visit. The odds of dying during the follow-up period were 8·5-fold higher among MSD cases compared to controls ($p<0·01$), with most deaths (87·9%) occurring during the first 2 years of life.

However, rotavirus did not appear to be associated with growth faltering or death. This is not unexpected in a health center-based study where life-saving fluid-resuscitation for dehydration is performed; infants with rotavirus not accessing care likely experience higher morbidity and mortality. Among those who died, the odds of having RV decreased exponentially over the ensuing days ($p<0.01$), suggesting that some RV-associated deaths may have occurred acutely, as would be expected with dehydrating diarrhea (**Figure 1**). On the other hand, pathogens such as *Cryptosporidium* and ST-ETEC were significantly associated with an increased risk of linear growth faltering and death during the 2-3 month follow-up period after an episode of MSD.

Figure 1. Duration of survival for children with and without rotavirus-associated MSD



As rotavirus vaccines are widely introduced into routine infant immunization programs across low-income countries during the next few years, facilitated by financial support from the GAVI Alliance, marked reductions in child deaths and hospitalizations from rotavirus diarrhea are anticipated. Because strategies that are effective for delivering oral vaccines to infants in wealthy countries may perform sub-optimally in resource-poor countries,[5-9] monitoring the impact and effectiveness of programmatic introduction of rotavirus vaccine in such settings is a high priority and efforts are ongoing. If the vaccine is shown to be effective, similar countries might be motivated to seek introduction; if effectiveness is not satisfactory, the findings must be scrutinized to determine the need to modify vaccination schedules or formulations to enhance the performance.

Several factors have been identified that may contribute to low vaccine efficacy in resource-poor countries, including interference from maternal antibody,[10] breastmilk,[11] co-administered oral polio vaccine,[12] undernutrition, environmental enteropathy, altered microbiome, and micronutrient deficiency.[13] A role for genetic determinants in vaccine response has generated increasing interest.[13] One unexplored mechanism is the effect of genetically determined gut histo-blood group antigens (HBGA) which mediate viral attachment to the intestinal epithelium. Synthesis of the relevant HBGAs is regulated by *FUT2* (Secretor) and *FUT3* (Lewis) genes.[14,15] Protection against severe rotavirus gastroenteritis has been demonstrated in U.S. children who had a genetic polymorphism that inactivates *FUT2* expression on intestinal epithelial cells.[16] Rotavirus serotype P[8], the prevalent circulating serotype in high-income settings, has been shown to exclusively infect children of Lewis-positive and secretor phenotypes in Burkina Faso and Nicaragua.[17] Interestingly, both globally used rotavirus vaccines include P[8] as the sole P serotype. If the current P[8] dominant vaccine virus cannot enter the host cells, because there are no appropriate HBGA receptors, a vaccine immune response is not stimulated. The individual is protected against the rotavirus P[8] serotype through innate resistance, but not protected with vaccine against other circulating rotavirus P serotypes. This would subsequently lower VE in regions where P[8] is not a prevalent serotype. In African countries there is both a higher prevalence of HBGA mutations and P[6] is a dominating rotavirus P serotype.[17-19] A nested study within VIDA will explore the relationship between genetic mutations in HBGAs and rotavirus vaccine failure.

Most certainly, the impact of rotavirus vaccine introduction on the epidemiology of diarrheal diseases in developing countries will not be limited to changes in rotavirus-associated morbidity and mortality alone. The entire landscape of pathogens associated with childhood diarrhea and its adverse outcomes will be altered. For example, in the U.S. and some middle income countries, norovirus, rather than rotavirus, is now the most common agent identified in children hospitalized with acute gastroenteritis since the introduction of rotavirus vaccine.[20,21] In developing countries, the vaccine impact will interact in a dynamic way with the complex array of factors already influencing the epidemiology of diarrheal diseases, including economic development, climate change, deforestation, emergency displacement, urbanization, access to HIV prevention and treatment, and availability of nutritious food, improved water and sanitation. It is possible that rotavirus vaccine introduction may have a profound impact on certain aspects of diarrheal morbidity and mortality, e.g., incidence and mortality related to acute dehydrating diarrhea, while producing little effect on other adverse clinical outcomes, e.g., linear growth faltering within 2-3 months after an episode of MSD. Without current epidemiologic information, our case management algorithms may be ineffective and we may miss opportunities to develop new interventions. For example, the importance of *Cryptosporidium* and ST-ETEC demonstrated in GEMS uncovered a need to develop point-of-care methods for diagnosis, prevention, and treatment of these agents, and to intensify strategies that address the nutritional consequences of diarrheal diseases. As we prepare for the era of widespread rotavirus vaccine uptake, continued progress in the control of diarrheal diseases will require a new foundation of data to develop and prioritize strategies that will ensure our efforts are relevant and appropriate to the causes and consequences of diarrheal diseases of the future.

This proposal aims to utilize the established GEMS infrastructure and methodology for conducting a 36-month case-control study of MSD in a censused population to provide a detailed assessment of the shifts in incidence, etiology, and adverse clinical outcomes of MSD that emerge in three prototypic low income countries in sub-Saharan Africa (Mali, The Gambia, and Kenya) once rotavirus vaccine has been introduced. The objectives of this study are:

4. Objectives

1. To assess the impact of rotavirus vaccine introduction on the:
    a. Etiology of MSD, by comparing the number and proportion of MSD cases with specific pathogens before rotavirus vaccine introduction (i.e., during GEMS) and in the study period, as well as comparing adjusted pathogen-specific incidence and attributable fraction;
    b. Adverse clinical consequences of MSD, measured as the linear growth attainment and mortality during a 2-3 month follow-up period after study enrollment. In addition, the frequency of persistent diarrhea (lasting 14 days or longer) will be measured using a self-administered Memory Aid developed for GEMS;
    c. Overall incidence of MSD; and
    d. As an exploratory objective, diarrhea- associated under-5 mortality in the DSS population measured using verbal autopsy.
2. To provide a well-characterized library of stool samples for analysis by TaqMan®, a highly sensitive, quantitative molecular assay to more precisely define the pathogen-specific diarrheal disease burden
3. To determine the effectiveness of a full-course of rotavirus vaccine using a case-control study design with two separate control populations: rotavirus test-negative cases with MSD, and matched

community controls. Additional information that can be gleaned from the case-control study includes:

a. Effectiveness of a partial course of rotavirus vaccine;

b. Duration of protection;

c. Presence of protection in children too young to receive vaccine (herd immunity); and

d. Potential risk factors for vaccine failure, including nutritional status, concomitant infection with enteropathogens that may modulate the immune system or the expression of diarrheal symptoms, such as *Giardia*, soil helminths, and *H. pylori.* Other factors could be examined under separate funding.

4. To test whether genetic mutations in histo-blood group antigens *FUT2* (secretor) and *FUT3* (Lewis) are associated with an increased risk of moderate-to-severe rotavirus diarrhea, among vaccinated children.

5. Study sites

This proposal aims to utilize the infrastructure and expertise for population-based surveillance refined during GEMS to measure the impact and effectiveness (both direct and indirect) of rotavirus vaccine introduction into the national immunization program. The Center for Vaccine Development (CVD) at the University of Maryland, Baltimore (UMB) will serve as the coordinating site. We have selected three GEMS sites in sub-Saharan Africa (Bamako, Mali; Basse, The Gambia, and Siaya County, Kenya) that share the observation in GEMS that rotavirus is the most important pathogen in during the first 2 years of life. All three countries recently introduced rotavirus vaccine but vary with respect to the vaccine introduced. Mali and The Gambia are using RotaTeq while Kenya introduced Rotarix. The sites all have moderate-to-high under 5 mortality rates, but differ with respect to setting (urban vs. rural), disease burden (rotavirus incidence and MSD-associated mortality rates), co-morbidities that could affect vaccine take (HIV and malaria transmission, malnutrition), and health indicators that might reflect healthcare quality and utilization (DTP3 coverage) (**Table 2**). Mali, in particular, is a paradigm of a landlocked least developed country with high childhood mortality (128 deaths under 5 years per 1,000 live births) that is not confounded by HIV co-morbidities (HIV adult prevalence ~1%). All three sites have an established record of performing outstanding field epidemiology and Phase 2-4 vaccine trials. There is an additional poignant rationale for measuring the impact and effectiveness of rotavirus vaccine introduction in Bamako. In a Phase 3 RotaTeq trial, while the African sites in Kenya and Ghana found a 1 year efficacy of 65% and 83%,

Table 2. Study sites

	Setting	RVV intro	% Adult HIV	Malaria transm[12]	U5MR[13]	% DTP3[13]	GEMS					
							DSS pop'n <5 yrs old	No. SHC	% improved drink H_2O	MSD CFR (%)	RV incid (95% CI)†	Enroll HAZ‡
Basse, The Gambia	Rural	Aug 2013	Low (1.3)[22]	High	73	98	28,898	6	45-46	3.8	3.2 (1.7-4.6)	-0.81
Bamako, Mali	Urban	Jan 2014	Low (0.9)[22]	High	128	74	32,526	9	68-71	1.1	8·4 (3·5-13·3)	-0.60
Siaya County, Kenya	Rural	July 2014	High (15.4)[23]	Mod	73	83	23,294	9	10-12	3.5	10·1 (5·4-14·8)	-0.96

RVV=rotavirus vaccine; DSS=Demographic surveillance system; MSD=moderate-to-severe diarrhea; CFR=case-fatality rate; SHC=sentinel health center; U5MR=mortality rate <5 yrs old per 1,000 live births; †Incidence per 100 child-years among infants 0-11 months in the site's demographic surveillance survey (DSS) population; ‡Mean HAZ=height for age z score at enrollment for MSD cases in the 0-11 month strata.

respectively, in Mali only 7 cases of rotavirus gastroenteritis that met the case definition of severe diarrhea were identified during the first year of the study, and thus a site-specific efficacy could not be determined with precision.[6] As plans for vaccine introduction elsewhere in sub-Saharan Africa unfold, it is essential to provide more clarity on this issue.

6. Study population (sampling frame)

Each site will provide a censused population, which will be updated using a demographic surveillance system (DSS) which is ongoing and not the subject of this protocol. The DSS provides the sampling frame from which all participants will be recruited. Field workers visit each household to update the census with births, deaths, and migrations at least twice annually. As in GEMS, these data will be supplemented using village reporters who reside in each village. The reporters will contact key individuals (third trimester pregnant women, elders, midwives, religious leaders, cemeteries, etc.) on a weekly basis to continuously update the DSS database with births and deaths among children <5 years of age. By maintaining an updated DSS database, and linking each member to an address, we will be able to: i) enumerate the population from which cases and controls are enrolled for calculation of incidence rates; ii) select age-stratified random samples of children to perform surveys for the purpose of determining the proportion of children with MSD who are taken to the SHCs when they develop MSD, so that incidence rates based on children who seek care at the SHCs for MSD can be corrected for the children who do not seek care at the SHCs. iii) perform vaccine coverage surveys of random samples of children for measurement of vaccine impact; iv) randomly select matched controls for each case of MSD enrolled.[24]

7. Informed consent

The clinical protocol and subsequent amendments must be approved by the Institutional Review Board (IRB) of the University of Maryland, Baltimore (UMB) in addition to the relevant local IRBs overseeing each site. UMB IRB requires that all written or taped documents that are presented in a language other than English be accompanied by a certificate from an independent observer verifying that the translation is true to the English consent form. The consent process will follow local customs, standards, and regulations. Often the first step is a process of community consent, during which the protocol is presented to local leaders at each site, followed by a question and answer period, discussion, and a decision about whether they approve the protocol.

Written, informed consent will be obtained from the parent or primary caretaker of each case and control participating in the Case-Control Study who meets all eligibility criteria before any research activities are performed. In either situation, the study will first be explained in local language or dialect. The parent or primary caretaker will be given a copy of the consent form to read or share with confidants who are able to read. When the parent or primary caretaker cannot read, the written consent form will be read aloud or recorded on audiotapes for the parents to hear. After completing these informational sessions, the parent or primary caretaker will be given ample opportunity to ask questions. Thereafter, consent will be documented by asking the parent or primary caretaker to sign his/her name (or place an “x” or a fingerprint if unable to sign his/her name) on the consent form. It was our initial intent to obtain an impartial third party to witness the consent process and sign the consent document if the parent/primary caretaker cannot read. However, in rural communities where adult literacy is low, we found it difficult in some instances to identify a literate witness in the participant's village. We had to search several villages to locate such an individual. The participant's family appeared uncomfortable that we brought a stranger into their home to witness consent. Therefore, we would like to propose for this minimal risk study that in the event that a literate impartial witness cannot be located, the consent process, be it reading the consent form to the participant's parent/caretaker or having them listen to audiotapes, will be documented by a

member of the study team. The person signing could be the same person who is obtaining the consent. The original signed/ imprinted form will be retained at the site. Providing a copy to the parent or primary caretaker will be at the discretion of the site or at the request of the parent. There will be separate consent forms for VIDA-plus cases and controls.

8. Assessing health care utilization and rotavirus vaccine coverage of resident children (HUCS)

8.1. Overview

The HUCS is conducted as part of the DSS protocol, not this protocol. The information is provided here because it will be linked to the data collected as part of this case-control study. At least twice a year, children 0-59 months of age will be selected from the DSS population at each site to participate in a **Health Care Utilization and Coverage Survey (HUCS).** In essence, the primary caretakers of participants will be asked additional questions during the routine DSS interview. The HUCS serves several purposes: i) to determine the proportion of children who do not seek care at the SHCs when they have MSD, which can be used to calculate population-based disease incidence estimates derived from children seeking care at the SHCs and adjusted for children who do not seek care at the SHCs for MSD; ii) to allow us to determine where children from the DSS population seek care when they have MSD, to guide the choice of SHCs for case enrollment during the case-control study; iii) to assess rotavirus vaccine coverage; and iv) to determine whether vaccinated and unvaccinated children differ with regard to their propensity to seek care at a SHC for MSD. Three age strata will be targeted: infants (0-11 months), toddlers (12-23 months), and young children (24-59 months).

8.2. Sampling

From the continually updated DSS database at each study site, computerized lists of children will be constructed for each of the age groups of interest (0-11, 12-23, and 24-59 months). The census database thus should be as current as possible, to include recent births and to assign children who have crossed an age group boundary to the appropriate group. The list will define the population from which the HUCS sample is drawn. Even with an updated list, there will be births between the time of preparation of the list and conduct of the survey, so that infants at the earliest ages will probably be somewhat underrepresented in the HUCS.

In Kenya, the HUCS questions will be administered to the primary caretakers of all age-eligible children during each DSS round. In The Gambia and Mali, a random selection of approximately 550 eligible children will be selected from the 0-11 month age group (over-sampled because of the potential difficulties locating children in this age group, e.g., because of aging beyond the strata between DSS rounds, as yet undetected births which occurred between DSS rounds, and higher mortality rates), and 500 eligible children from each of the two older age strata will be selected (12-23 months and 24-59 months) using the updated census list. We anticipate that approximately 10% of each sample (almost 20% of infants) will not be evaluable. Possible reasons for ineligibility include aging out of the age group for which the list is prepared, some of which will occur in spite of updating the census list; a child no longer living, or miscoded as living, in the HUCS area; death of a child; and errors in the census and/or surveillance.

When the DSS team identifies an ineligible child in the HUCS sample, that child will be deleted from the sample and the census/surveillance list for that age group, to make the list as accurate as possible. **If the interviewer finds that a child falls outside his/her assigned age stratum but is nonetheless <60 months of age, the interview will be performed. If the child is ≥60 months old, s/he will be considered ineligible and the interview will not be conducted.** For The Gambia and Mali, we anticipate a final sample size within each age stratum of about 450 children per round, for a total of approximately 2700 children per age

stratum over 6 rounds. Assuming the same population sizes in Kenya as in GEMS, we expect to include totals over 6 rounds of approximately 22,600 children 0-11 months of age, 23,300 children 12-23 months of age, and 69,900 children 24-59 months of age.

If information for a child in the HUCS sample cannot be obtained after three attempts by the interviewer, but the child is considered eligible according to age and location of residence, that child will be kept in the sample and considered a non-responder. If the child is eligible but the primary caretaker refuses to participate, the child will be kept in the sample and considered a 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. To perform this weighted analysis, the site will save the DSS dataset each time a survey is performed. They will record the number of children in the DSS population from which the HUCS sample was chosen who belong to each age stratum, by gender. These data will be sent to the data coordinating center for use during analysis to weight the HUCS sample according to the DSS population.

To summarize, the following constitute eligibility criteria for the HUCS:

8.2.1. Inclusion

1. Age 0-59 months

2. Belongs to the DSS

3. Represented in the database (Kenya) or randomly selected from the DSS database (Mali and The Gambia)

8.3. Contacting a selected child

The HUCS will be conducted by the DSS team during their routine rounds. The respondent will be the child's primary caretaker. If a primary caretaker is not available, this will be recorded and the interviewer will try to leave a message indicating when the interviewer is likely to return. A total of 3 attempts will be made to contact a primary caretaker, after which time the child will be considered a nonresponder.

8.4. Consent issues

The HUCS is considered to be part of the DSS at each site since the questions are similar in nature, brief, and carry the same risks and benefits as the questions already being asked during the DSS. Therefore, separate consent is not performed. The procedures are described here because the data will be shared.

8.5. HUCS questionnaire

The parent/primary caretaker of each selected child will be asked whether the child had diarrhea in the previous 7 days. If so, they will be asked a brief panel of ~30 questions such as: i) whether the child had: sunken eyes (more than usual), wrinkled skin, IV hydration, dysentery, or was hospitalized; ii) whether they took the child for care outside of the home, and if so where. Information will be solicited about the household and family composition, household possessions (to calculate a wealth index as a proxy for socioeconomic status),[25] occurrence and nature of recent diarrheal illnesses among children younger than 5 years, and health care utilization practices. The survey may be modified as appropriate for each site, while adhering to the main elements. When birth date is not known and it may be necessary to use an events calendar to estimate ages. The first page of the questionnaire will contain identifying information; this page will be kept locally at each site; the remaining pages of the form containing the study number and information obtained will be transmitted to the central data center.

The effect of distance from the health center as well as topologic barriers on health care utilization will be analyzed. Therefore, the location of the household within the census tract will be of interest. To collect

this information, a log will be maintained linking the child's HUCS study identification number with data from the census such as the child's census ID number, village name, census cluster number, compound number, household number, and global positioning system (GPS) coordinates. Although no names will be recorded, this information could potentially be linked to a child's identity. Therefore, the following precautions will be taken to maintain confidentiality: the log will be transmitted to the data coordinating center via email separate from the clinical and epidemiologic information that is collected and will be stored at the central data coordinating center in a secure location, separate from the case report forms.

An important component of the HUCS will be determination of the child's vaccine coverage. At each survey, vaccination history will be obtained from children who are age-eligible to have been vaccinated (for rotavirus vaccine this will take into consideration the date that the vaccine was introduced into the Expanded Programme on Immunization (EPI) centers serving their DSS population. We will ask to see the child's vaccination card and record the dates that the child received vaccines against rotavirus, oral polio, and diphtheria-pertussis-tetanus containing vaccine, the EPI center attended. We will also retain a photocopy, scanned image, or photograph of the vaccination card. If the vaccination card has not been retained, then we will attempt to verify the child's vaccination status at the vaccine administration center and retain a photocopy, scanned image, or photograph of the clinic record. These data will provide information about health care utilization patterns for diarrhea among children according to whether they received rotavirus vaccine.

9. Case-control study to measure rotavirus vaccine impact on diarrheal disease etiology and rotavirus vaccine effectiveness

9.1. VIDA case recruitment

A case-control study will be undertaken to determine the impact of rotavirus vaccine introduction on the incidence, etiology, and adverse outcomes of MSD. Each site will identify sentinel health centers (SHCs) where children 0-59 months of age from their DSS seek care when they experience MSD. Using GEMS methodology, we will identify all DSS children at each site ages 0-59 months and seeking care at a SHC for diarrhea.[26] A study clinician will evaluate each child with diarrhea for eligibility. Eligible episodes must be new (onset after $\geq$7 diarrhea-free days), acute (onset in the previous 7 days), and fulfill $\geq$1 of the following criteria for MSD: 1) sunken eyes (confirmed by parent/caretaker as more than normal); 2) loss of skin turgor (abdominal skin pinch with slow (but $\leq$2 seconds) or very slow (>2 seconds) recoil); 3) intravenous hydration administered or prescribed, 4) hospitalized with diarrhea or dysentery, or 5) dysentery.[24] To ensure even sampling throughout the year, the sites will each enroll the first ~8-9 eligible cases per age stratum (0-11 months, 12-23 months, and 24-59 months) per fortnight throughout a 36 month enrollment period, for a total of up to 660 children (220 per age stratum) per site per year, and 1,980 children (660 per age stratum) per site over 3 years. In Kenya, where the first year of enrollment was low, cases may be enrolled in excess of 9 per fortnight to make up for previous low enrollment.

9.2. VIDA-plus case recruitment

To ensure sufficient power to assess rotavirus vaccine effectiveness, we will use a sampling scheme in which all children with MSD will be enrolled during rotavirus season, but the children who are age-eligible to receive rotavirus vaccine and are enrolled in excess of 9 per age stratum per fortnight will be tested only for rotavirus. We have observed in the GEMS that there are ample additional MSD cases during rotavirus season to expand recruitment numbers. In Kenya, there are no seasonal peaks, so additional enrollments could be continued year round. In Mali, we will enroll children in VIDA-Plus year round to ensure that all rotavirus cases are captured. **VIDA-plus cases and their controls will not be required to complete the memory aid or undergo a 60-day follow-up visit. They will, however, provide a saliva sample.**

9.3. Case eligibility criteria (for VIDA and VIDA-Plus)

Children who meet the inclusion criteria will be considered as "Eligible MSD cases" for the purpose of the analysis, even if they also meet exclusion criteria.

9.3.1. Case inclusion criteria

1. 0-59 months of age
2. Resides in the demographic surveillance system (DSS) catchment area
3. Seeking care at a sentinel health center (SHC) attached to the DSS
4. Diarrhea, defined as 3 or more loose stools within the previous 24 h
5. The diarrheal episode began at least 7 days since the last occurrence of diarrhea[27,28]
6. The onset of diarrhea was no more than 7 days before study enrollment
7. The diarrhea meets at least one the following criteria for "moderate-to-severe":
    a. Sunken eyes, more than normal
    b. Loss of skin turgor
    c. Intravenous rehydration administered or prescribed
    d. Dysentery (diarrhea with visible blood in stool)
    e. Hospitalized with diarrhea or dysentery
8. To be eligible for saliva collection a case must be 3-23 months of age

Notes -- The following criteria are acceptable for enrollment as a case during a new episode of MSD as long as the selection of that case followed the site's sampling method:

- Served previously as a case and completed 60-day follow-up visit
- Served previously as a case in the VIDA-plus component
- Served previously or currently as a control in either VIDA or VIDA-Plus

9.3.2. Case exclusion criteria

1. Currently enrolled as a case (currently means that the child is enrolled and has not yet undergone a 60-day follow-up visit)
2. Failed to provide a sufficient stool sample ($\geq$4 grams/size of 4 peas) within allowable time period (For VIDA-plus, $\geq$2 grams, or 2 peas, is required)
3. A rectal swab was not obtained prior to antibiotic receipt in the health center (not applicable for VIDA-plus)

9.4. Characterizing case eligibility

The following information will be tracked in the **Case Registration and Eligibility Logs** for the purpose of understanding bias that could confound subject selection. This information also will allow the denominators to be adjusted to derive population-based estimates of moderate-to-severe diarrhea and other study endpoints in each age stratum (0-11 months, 12-23 months and 24-59 months):

- Total number of visits to hospital or health center.
- Total number of admissions (if the center has inpatient facilities).
- Total number of admissions/visits with diarrhea/dysentery:
    - Eligible to enroll (meets inclusion criteria for MSD)
        - Enrolled
        - Not enrolled
            - 14 day quota filled (unless VIDA-plus is ongoing)
            - Child died before invitation

- Child's severe condition did not allow time for enrollment process
- Caretaker not available
- Sufficient stool sample not provided:
  - ❖ For VIDA: ≥4 g/size of 4 peas) within allowable time period
  - ❖ For VIDA-plus: ≥2 grams/size of 2 peas) within allowable time period
- For VIDA: a rectal swab was not obtained prior to antibiotic receipt in the health center
- Refused
  - ❖ Parent/caretaker too busy
  - ❖ Does not like research
  - ❖ Child too sick
  - ❖ Other (specify)

➢ Ineligible
- o Fails to meet the inclusion criteria (described in the next section)
- o Currently enrolled as a case (meaning a 60-day follow-up has not been performed, if applicable)
- o For VIDA-plus: not age-eligible to receive rotavirus vaccine
- o Other (specify)

9.5. Control recruitment

For each child with diarrhea enrolled in the study, one to three healthy control children will be randomly selected from the community or village in which the case resides. The control will be matched to the case by age, gender, and time that the index case presented. A list of a minimum of four potential controls, if at least four can be identified, will be generated by computer from the demographic surveillance database. A field worker will first visit potential controls until a control is identified who is eligible, agrees to participate, and is able to provide an adequate (at least 4 grams) whole stool in a timely fashion. The **Memory Aid** (**Figure 2**) described below will be used to determine whether a control goes on to develop diarrhea within 7 days of presentation of the index case. We will perform a subanalysis excluding controls who develop diarrhea within one week after enrollment to determine whether the attributable fractions of pathogens change. The strategy for age matching controls to cases is shown in **Table 3**.

Table 3. Allowable age range (in months) of matched controls by age of index case

Index Case	Matched control LL	Matched control UL	Index Case	Matched control LL	Matched control UL	Index Case	Matched control LL	Matched control UL	Index Case	Matched control LL	Matched control UL	Index Case	Matched control LL	Matched control UL
0	0	2	**12**	12	16	**24**	24	28	**36**	32	40	**48**	44	52
1	0	3	**13**	12	17	**25**	24	29	**37**	33	41	**49**	45	53
2	0	4	**14**	12	18	**26**	24	30	**38**	34	42	**50**	46	54
3	1	5	**15**	12	19	**27**	24	31	**39**	35	43	**51**	47	55
4	2	6	**16**	12	20	**28**	24	32	**40**	36	44	**52**	48	56
5	3	7	**17**	13	21	**29**	25	33	**41**	37	45	**53**	49	57
6	4	8	**18**	14	22	**30**	26	34	**42**	38	46	**54**	50	58
7	5	9	**19**	15	23	**31**	27	35	**43**	39	47	**55**	51	59
8	6	10	**20**	16	23	**32**	28	36	**44**	40	48	**56**	52	59
9	7	11	**21**	17	23	**33**	29	37	**45**	41	49	**57**	53	59
10	8	11	**22**	18	23	**34**	30	38	**46**	42	50	**58**	54	59
11	9	11	**23**	19	23	**35**	31	39	**47**	43	51	**59**	55	59

LL=lower age limit; UL = upper age limit (in months)

9.6. VIDA-plus control recruitment

For each VIDA-plus case, we will attempt to enroll 3 control children using the same methods as described above. **Unlike standard controls, VIDA-plus control children will not submit a stool sample, complete a memory aid, or undergo a follow-up visit. They will, however, provide a saliva sample.**

9.7. Characterizing control eligibility

The following information will be tracked for each index case using a **Control Registration Log**:

- Total number of controls identified by computer
- Total number of households approached
- The number of times a household was revisited if the primary caretaker was not home
- Total number of eligible controls
- Total number of controls invited to participate
- Total number of controls enrolled
- Reasons for non-enrollment
  - Ineligible (family not living at address, child died, does not meet eligibility criteria)
  - Eligible but not enrolled
    - Not contacted because requisite number of controls was already enrolled
    - Caretaker or child not present
    - Caretaker refused
      - Parent/caretaker too busy
      - Does not like research
      - Child too sick
      - Other (specify)
    - Child did not provide adequate stool sample ($\geq$ 4 grams/size of 4 peas) in required time period
    - Other (specify)

9.8. Control eligibility criteria

For each case enrolled, we will aim to enroll 1-3 children without diarrhea during a home visit, following an algorithm that increases the requisite controls according to the number of cases enrolled in that fortnight.[24] Controls matched to each individual case by age ($\pm$2 months for cases aged 0-11 months and $\pm$4 months for cases aged12-23 and 24-59 months), gender, and residence (same or nearby village or neighborhood as the case) will be randomly selected from the site's DSS database and enrolled within 14 days of the case. Potential controls who had diarrhea in the previous 7 days are ineligible.

9.8.1. Control inclusion criteria (for VIDA and VIDA-Plus)

1. Resides in the demographic surveillance system (DSS) catchment area
2. No diarrhea within 7 days of enrollment*
3. Age-matched to index case as follows: $\pm$2 months for cases 0-11 months, and $\pm$4 months for cases 12-59 months. The matched control may not exceed the stratum boundaries of the case, e.g., a control for an 11 month old case must be between the ages of 9 and 11 months and a control for a 13 month old must be between the ages of 12 and 17 months (**Table 3**)
4. Same gender as case
5. Same or nearby village or community as case**

6. Time: enrolled within 14 days of presentation of the index case
7. To be eligible for saliva collection a child must be matched to a case who is eligible for saliva collection

Notes:

* Control children are included in the analysis irrespective of whether they developed diarrhea after enrollment.

**Each site will follow an algorithm beginning with the case's village/neighborhood, and then proceeding to villages/neighborhoods located at an increasing distance from the case's village/neighborhood until a control can be identified.

9.8.2. Control exclusion criteria

1. Currently enrolled as a case or control (currently means that the child is enrolled and has not yet undergone a 60-day follow-up visit)
2. Fail to provide a sufficient stool sample ($\geq$4 g/size of 4 peas) within allowable time period (not applicable for VIDA-plus controls)

9.9. Enrollment visit: Data collection from cases and controls

After informed consent has been obtained, the investigator will administer a standardized questionnaire to the parent/primary caretaker of cases and controls. The enrollment visit will occur in the health center for cases and in the home for controls. The questionnaire will include the following information (note that diarrheal history, physical examination, and visit outcome data will be collected only for cases):

9.9.1. Identifying information

Data collected at enrollment will include the child's name, address, and other identifiers that will permit study personnel to perform a home visit 60 days after enrollment, as well as the child's study number and initials. This form will remain at the site under secure conditions and will not be transmitted off-site to the central database. Only authorized personnel will have permission to access this information.

9.9.2. Demographic/Clinical/Epidemiology information

Approximately 60 questions will be asked concerning demographic, epidemiologic, and clinical characteristics. The child's age will be determined. Accuracy of age estimates is critical to assessments such as height and weight z scores. In some field sites (particularly in sub-Saharan Africa), birth date may not be known and it may be necessary to use an events calendar to estimate ages. Information on household possessions, household construction, and parental educational attainment will be used to assess demographic factors that have been shown to be correlated with child survival and to calculate a wealth index as a proxy for socioeconomic status.[25] Questions will include household size and composition, cooking fuel, and access to improved water and sanitation, and breast feeding.

9.9.3. Anthropometric measurements

The study team will measure the height, weight, mid-upper arm circumference (MUAC), respiratory rate, capillary refill time, and axillary temperature. The height, weight and MUAC will be measured for each case and each control at enrollment and again 60 days later. Height and MUAC will be measured thrice at each visit and the average value will be calculated during analysis. Methods are adapted from *How to Weigh and Measure Children: Assessing the Nutritional Status of Young Children in Household Surveys*, United Nations Department of Technical Cooperation for Development and Statistical Office, 1986. The

height of children 0-23 months of age or those too ill to stand will be measured (to the nearest 0.1 cm) in the recumbent position using a board with a fixed head and sliding foot piece. For children 24 months and older who can stand alone, a standing height will be measured (to the nearest 0.1 cm). Weight will be measured to the nearest 0.1 kg using a digital scale that is calibrated daily. Each of the indices will be expressed in standard deviation units (SD) from the mean of the NCHS/CDC/WHO International Reference Population.

9.9.4. Vaccination information

For children age-eligible to have received rotavirus vaccine, we will ask to see the child's vaccination card and record the dates that the child received rotavirus vaccine. Data on diphtheria-pertussis-tetanus containing vaccine and polio vaccine will also be collected. We will also retain a photocopy, scanned image, or photograph of the vaccination card. If the vaccination card has not been retained, then we will attempt to verify the child's vaccination status at the vaccine administration center and retain a photocopy, scanned image, or photograph of the clinic record.

9.9.5. Physical examination (cases only)

A study clinician will perform a focused physical examination to assess the child's vital signs, hydration and nutritional status. He/she will record observations about any stools that are passed and will measure the child's axillary temperature and respiratory rate.

9.9.6. Outcome of enrollment visit (cases only)

The study team will follow the child's clinical status throughout his/her stay in the outpatient clinic or hospital to document events that might include the treatment prescribed and received for the diarrheal illness and the child's clinical status upon discharge.

9.9.7. Obtaining stool samples from cases and controls

A single, fresh, whole stool specimen will be collected from both cases and controls at enrollment, **with the exception of controls for VIDA-plus c**ase**s**. For cases, the specimen must be collected within 12 hours of registration at the SHC. Arrangements can also be made to collect the specimen from the home during that time period if necessary. For controls, the specimen will be collected at the home.

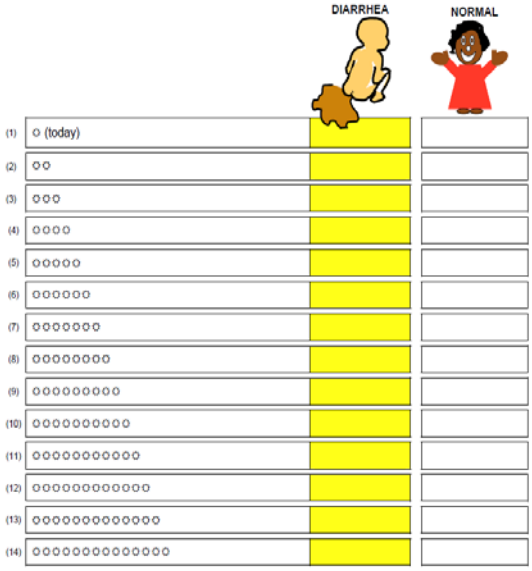
A rectal swab will be obtained from any VIDA MSD case IF antibiotics will be administered imminently. (**Rectal swabs are not necessary for VIDA-plus cases).** Nonetheless, a child who provides a rectal swab also must provide a whole stool within 12 hours of registration to be analyzable in the study. The fact that the stool will be collected after administration of antibiotics should not affect the multiplex PCR and RT PCR assays for viruses or immunoassays for viral and protozoal antigens. However, the antibiotics will adversely affect the laboratory's ability to isolate various bacterial pathogens. This strategy will permit collection of an adequate sample for bacteriology prior to antibiotic administration as well as a whole stool for identification of pathogens that are best detected in whole stool but are not expected to be affected by antibiotic administration. In Mali, if a child receives antibiotics prior to a rectal swab being collected AND the child would otherwise be eligible for VIDA-Plus, the child may be enrolled in VIDA-Plus.

9.9.8. Memory aid to track occurrence of diarrhea

At the enrollment visit, the primary caretaker of cases and controls (**with the exception of cases and controls in VIDA-plus**) will be given a card and supplies to record whether the child experiences diarrhea for the 14 days after enrollment (**Figure 2**). To allow illiterate parents/caretakers to complete the form, the form will be pictorial and a different symbol will be shown to record whether there is diarrhea or not on a

given day. The parent/caretaker will be encouraged to involve any school children in the household in the process. A diarrheal day is defined as a day with 3 or more loose stools. The episode ends when 7 consecutive days pass without diarrhea. This instrument will be critical for detecting cases of persistent diarrhea.

Figure 2. Memory Aid. For 14 days after enrollment, the primary caretaker places an "x" in the corresponding box to indicate whether the child had diarrhea (≥3 abnormally loose stools) or normal stools



9.9.9 Obtaining saliva sample from eligible VIDA and VIDA-Plus cases and controls

A saliva sample will be collected from eligible VIDA cases and their matched controls. For cases, the specimen is collected at the SHC, unless the child is too dehydrated to provide a saliva sample in which case the saliva may be collected after rehydration or at the 60 day follow-up visit. For controls, the specimen will be collected at home. If possible, we will wait at least 10 minutes after feeding to collect saliva. If sufficient saliva is not collected at the enrollment or follow-up visit, field staff may attempt a third saliva collection visit within 120 days of enrollment.

9.10. Data collection at the 60 day follow-up visit

Approximately 60 days after enrollment (range, 50 to 90 days), a field worker will visit the home of each case and control (**with the exception of cases and controls in VIDA-plus**). A standardized questionnaire will be administered to cases and controls to ascertain the vital status and health of the child. The **Memory Aid** will be reviewed with the parent or primary caretaker. Missing or unclear markings will be resolved, and the interviewer will sign and date the form. The child's axillary temperature will be recorded, as will the child's height, weight, and MUAC. Standardized observations about water and sanitation facilities will be performed. A third attempt at saliva collection may be made after the 60 day follow-up visit, within 120 days of enrollment.

9.11. Verbal autopsy (VA) in the event that a child dies during the follow-up period

If a child dies while in the hospital or health center, if the parent/primary caretaker reports that the child has died when the ~60-day visit is made, or if a death is detected during ongoing demographic surveillance, then information on the cause of death will be collected in a standardized fashion from the medical chart, the health care provider, and, if available, the death certificate. The DSS team at the site will be notified to perform a verbal autopsy (VA), which is a routine component of the DSS at each site.[29,30] VA is a method used to ascertain cause of a death based on an interview with parent or other caregivers using a standardized questionnaire that elicits information on signs, symptoms, medical history and circumstances preceding death. The cause of death, or the sequence of causes that led to death, are assigned based on the data collected by a questionnaire and any other available information. Rules and guidelines, algorithms or computer programs, may assist in evaluating the information to determine the cause of death. The main objective of VA is to describe the causes of death at the community level or population level where civil registration and death certification systems are weak and where most people die at home without having had contact with the health system. A standard VA instrument comprises a VA questionnaire, a list of

causes of death or mortality classification system, and sets of diagnostic criteria (either expert or data derived algorithms) for assigning causes of death.

9.12. Stool specimen collection and processing

9.12.1. Whole stool samples

The intent is to obtain at least 10g of stool; however, to be considered acceptable, the sample can weigh as few as 4 grams or 4 ml, or as an approximation resembles the size of 4 peas. Study staff will leave a Styrofoam container containing a cold pack at the site (if at home or in a health care facility lacking a specimen refrigerator). The collector, i.e., individual collecting the stool will either place a diaper in a sealed plastic bag or scoop whole stool into a ~100 ml specimen cup (the cup should be no more than ½ full). The collector will immediately place the specimen into either a designated specimen refrigerator or a Styrofoam container containing a cold pack and transported to the laboratory in the time frame specified by the laboratory Manual of Procedures.

When study staff in the field retrieve the stool specimen, they will immediately process it as follows:

1. Insert a cotton swab into the specimen (if dysentery is present, the swab should be inserted into an area of blood or mucus); the soiled swab should then be inserted into a screw top plastic vial containing modified Cary Blair transport medium.
2. Insert a second stool swab into the specimen (if dysentery is present, the swab should be inserted into an area of blood or mucus), and place the soiled swab in a screw top vial containing Buffered Glycerol Saline (chilled, if possible).
3. Place at least 10 g (equivalent to ~10 ml) of whole stool into an empty vial (the stool that is already in the specimen container can be used for this purpose if available); a minimum of 4 grams (~4 ml) is acceptable for VIDA, and 2 grams for VIDA-plus.
4. Put the vials in a sealed bag labeled with the child's study number, date and time of collection.
5. Place the bag immediately into either a specimen refrigerator or a Styrofoam container containing a fresh cold pack.
6. Deliver the specimen to the laboratory and plate within 18 hours of processing.

9.12.2. Rectal swabs (pertains only to selected cases, and not controls)

Each rectal swab will be moistened by dipping it in the medium that will be used for transport. The cottontip will be gently inserted into the child's rectum and rotated 360°. A properly collected rectal swab is stained or covered with fecal material. Two swabs may be inserted into the rectum simultaneously. The swabs will be processed and maintained at the temperatures described in the Manual of Procedures. The specimen will be delivered to the laboratory and plated within 18 hours of processing. Upon receipt in the laboratory, the specimens will be examined for acceptability: discoloration from fecal material, proper labeling, sealed containers (no leaks or cracks), and satisfactory low temperature of the transport container. The two swabs will then be sent to the microbiology bench for detection of bacterial pathogens.

Every attempt will be made to provide the results of cultures for bacterial pathogens to health care providers in a timely fashion. Children will be managed according to the standard of care at the SHC where they are seeking care.

9.13. Saliva sample collection and processing

Sufficient saliva will be collected by inserting a Salivabio (Salimetrics) Infant's cotton-tipped swab under the child's tongue or inside the cheek for a few minutes, as described in the saliva collection SOP. The swab

will be immediately stored at 4°C or on a cold pack for no more than 12 – 18 hours and taken to laboratory for freezing at -80°C. Samples will be shipped to University of Maryland for ELISA assays.

10. Laboratory methods

10.1. Specimen accession

Each fecal specimen will comprise (a) a whole stool specimen (in screw top fecal specimen cups carried in Styrofoam boxes with cold packs), (b) a fecal swab in Modified Cary Blair medium in a plastic screw top test tube, and (c) a fecal swab in buffered glycerol saline in a screw top test tube. Each specimen will be bundled and each labeled with the subject's identification number, date and time of collection. Upon receipt in the laboratory, the specimen number will be entered into the computer database and a laboratory fecal specimen report form will be labeled with the subject's study number. The specimens will be examined for acceptability: sufficient volume (ideally 10 ml or grams with a minimum of 4 ml or grams for VIDA, and 2 grams for VIDA-plus), proper labeling, sealed containers (no leaks or cracks), and satisfactory low temperature of the transport container. The specimen will be examined for volume, consistency (standard 1-5 grade), presence of gross blood, pus, or mucus.

Upon accession, the specimens will be aliquoted into the following containers:

1. ~1 gram of stool will be aliquoted to a single freezer vial, labeled with patient ID, for later processing for protozoal pathogens by immunoassay. The specimens will be placed at –20 ^{o}C immediately after accession.
2. ~1 gram will be aliquoted to a single freezer vial, labeled with patient ID, for later processing for viral pathogens by immunoassay. The specimens will be placed at –20^{o}C immediately after accession.
3. ~1 gram will be aliquoted equally to two freezer vials (i.e., 0.5 grams per vial), labeled with patient ID, for later processing with tests not available at time of the study.
4. Remaining stool will be sent to the microbiology bench for detection of bacterial pathogens.

10.2. Detection of enteropathogens.

Enteropathogens will be identified at each site using the uniform methods of GEMS with the addition of the modified *E. coli* evaluation from GEMS-1A.[31] Bacterial agents (*Salmonella*, *Shigella*, *Campylobacter*, *Aeromonas,* and *Vibrio* spp.) will be detected using conventional culture techniques.[31] Three putative *E. coli* colonies from each stool will be pooled and analyzed by a multiplex polymerase chain reaction (PCR) that detect targets for enterotoxigenic, enteroaggregative, enteropathogenic, and enterohemorrhagic *E. coli* (ETEC, EAEC, EPEC, and EHEC, respectively). The following gene targets define each *E. coli* pathotype: ETEC (either *eltB* for heat-labile toxin [LT], *estA* for heat-stable toxin [ST], or both), ST-ETEC (either *eltB* and *estA,* or *estA* only), typical EPEC (*bfpA* with or without *eae*), atypical EPEC (*eae* without either *bfpA, stx1*, or *stx2*), EAEC (*aatA, aaiC*, or both), EHEC (*eae* with *stx1* and/or *stx2* and without *bfpA*). Additional *E. coli* PCRs include an *E. coli* duplex PCR (primers to detect STp and *eae*), a *bfpA* monplex PCR and *E. coli* multiplex PCR #2 (primers to amplify stx1, stx2, *eae*, *efa-1* and *sen*). Commercial immunoassays will detect rotavirus (Elisa ProSpecT Rotavirus kit, Oxoid, Basingstoke, UK) and adenovirus (ProSpecT Adenovirus Microplate); adenovirus-positive samples will be tested for enteric adenovirus serotypes 40 and 41 (Premier Adenoclone kit, Meridian Bioscience, Cincinnati, OH, USA). Norovirus (genotypes I and II), sapovirus, and astrovirus will be detected using multiplex reverse transcriptase PCR.[31] Commercial immunoassays (TechLab, Inc., Blacksburg, VA, USA) will detect *Giardia lamblia*, *Entamoeba histolytica* and *Crytosporidium* spp. Two additional assays will be performed off-site. *Shigella* isolates will be serogrouped and serotyped at CVD.

ETEC isolates will be sent to the laboratory of Dr. Roberto Vidal at the University of Chile for PCR testing to determine fimbrial adhesion profiles.

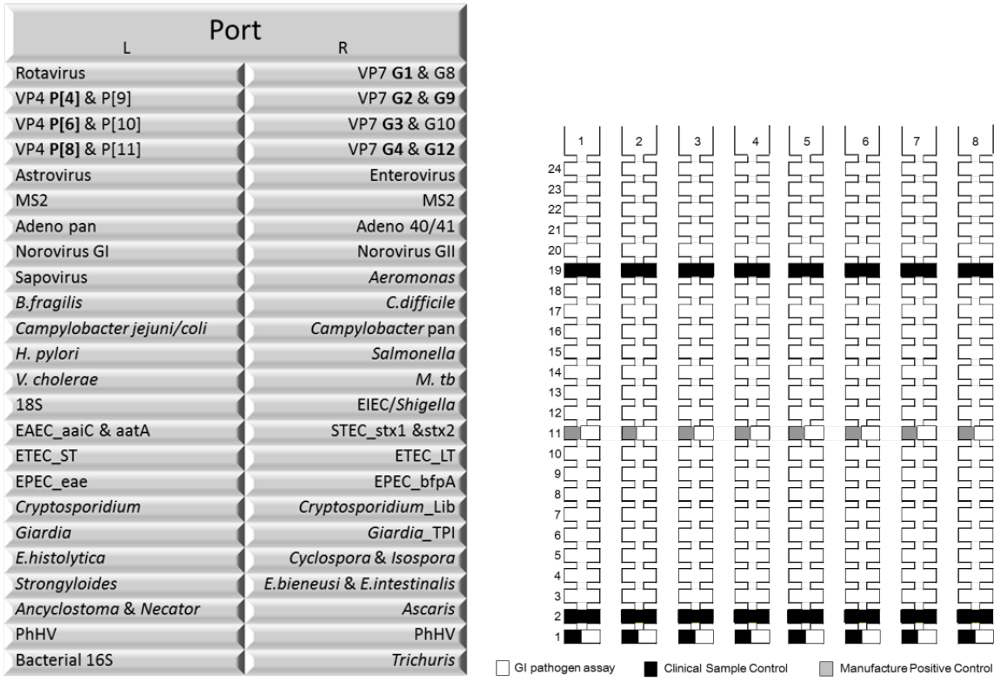
10.3. Future quantitative molecular assays using TaqMan® Array Cards (TAC)

Nucleic acids from stool samples will also be tested under separate funding using a quantitative molecular assay called TaqMan® Array Cards (TAC) (LifeTechnologies, VA).[32] The TAC assay will amplify nucleic acid for 26 enteropathogens: including multiple genotypes of several pathogens (**Figure 5**). Since Kenya and The Gambia already have this methodology in place, nucleic acids will be extracted on site from a 200 mg aliquot of stool and tested by TAC (under separate funding and in collaboration with Dr. Eric Houpt, UVA). It is anticipated that this technology will be established in Mali in the near future under separate funding. If that is not possible, then the CVD will extract nucleic acids from Malian stool samples according to Dr. Houpt's SOP and send an aliquot to UVA for testing by TAC.

10.4. Contributions to the CVD Specimen Repository

If the amount of stool collected is sufficient, at least 1 g of stool that remains after all assays are completed will be sent to the central repository at the CVD in Baltimore, MD and 1 g will remain at the site in -80°C freezer storage. Bacterial specimens will also be sent to the central repository at the CVD. Shipments to CVD will be made twice per year, with an extra "mop-up" shipment sent at the end of the study.

Figure 5. Enteropathogen Surveillance TAC



10.5. Detection of Lewis phenotype and Secretor status

Upon receipt in the laboratory, the specimen number will be entered into the computer database and a laboratory saliva specimen report form will be labeled with the subject's study number. Lewis phenotype and secretor status will be measured using ELISA

11. Data outcomes and analysis

11.1. Health care utilization and coverage survey

Outcomes. The questionnaire is designed to permit determination of the total number of children under 5 years of age, the number who met our criteria for MSD within the past 7 days, whether they sought care and where, and the number who ultimately received care within the first 7 days of illness at one of the SHCs where cases in the case-control study are recruited. This information allows serial determination of:

- Rotavirus vaccine coverage in a random sample of the DSS population;
- The relationship between vaccine coverage and health care utilization for MSD;
- The ability of the SHCs at each site to capture the cases of MSD within the 7-day eligibility period;
- Whether there are other SHCs commonly visited by children 0-59 months in the DSS when they have MSD that would be useful to include in the case-control study; and
- The true incidence of MSD during the study period.

Analysis. A reported episode of diarrhea will be considered as "MSD" if there are 3 or more loose stools in a 24 hour period of <7 days duration and one or more of the following three criteria are present:

1. One or more of the following indicating moderate-to-severe dehydration: sunken eyes (more than normal), loss of skin turgor (using "wrinkled skin" as a proxy), or intravenous rehydration;
2. Dysentery (diarrhea with visible blood in stool); or
3. Hospitalization with diarrhea or dysentery

The analysis will be performed as follows. A weight will be assigned to each responder – i.e., to each child in the sample for whom information is actually obtained. If the survey information is only partially complete for a child, that child will be considered a responder only if sufficient information is available to determine whether or not the child had MSD and, if the child had MSD, the number of days the episode had lasted and whether or not the child was taken to one of the study site SHCs. The weight assigned each child will represent the number of children in the population represented by the child. Weights will be calculated within each study site by age group and sex. Within each site-age-sex category, the weight for each responder will be the population total in that category divided by the number of HUCS responders in the category. Thus, the sum of the weights for responders in the survey sample will be the total number in the population in that category. In this way, weights will be adjusted within each category for non-response. Associations of HUCS variables with the probability of seeking care at a study health care facility will be assessed using logistic regression models. In these models, seeking care will be the dependent variable and other variable(s) of interest (gender, etc.) will be independent variables.

Within each site-age-sex or site-age category, the population total with a characteristic of interest is by definition the sum of the weights for HUCS responders in the category who have the characteristic. For example, let X and Y be estimated population totals within a category for the number of children with MSD and the number of those who receive care at a study health care facility within 5 days of onset of the diarrheal illness. Let R be the estimated proportion of children with MSD in that category who receive care at a study health care facility; then R = Y/X.

In order to estimate a confidence interval (CI) around R as defined above, e.g., a 95% CI, a jackknife procedure will be used for estimating variance. In such a procedure, an appropriate number of unique subsamples (e.g., 200) will be drawn for each age group or smaller category. One way to form a subsample is by deleting one or more randomly chosen observations from the original sample, including non-responders in the original sample, and taking what is left as the subsample. For each subsample, weights will be calculated and R obtained as though the subsample were the entire original sample. The variance of

R in the entire original sample will be estimated from the R's for the subsamples. The 95% CI for the population value of R will be $R \pm 1.96$ s, where s is the square root of the variance estimated by jackknife as described above. This assumes that the ratio R is approximately normally distributed.

11.2. Impact of vaccine introduction on the overall etiology of MSD

Outcomes. When the microbiological analyses are completed, the proportion of cases that yield a specific pathogen will be known for each 2-week and 4-week period. In data analysis, this proportion will be applied to the total number of MSD cases seen in that time period (or if applicable, by month) to estimate the total number of MSD cases with that pathogen. The following endpoints can be derived and compared pre- and post-rotavirus vaccine introduction to assess the impact of vaccine introduction:

- The number of MSD cases;
- The number (%) with each of the major pathogens significantly associated with MSD by conditional logistic regression;
- The incidence of overall and pathogen-specific MSD; and
- The attributable fraction of each major pathogen, by age stratum.

Analysis. A systematic measurement of the burden of MSD using a case-control study design was conducted during GEMS for 4 years at each site (with a 10-11 month hiatus between years 3 and 4). These data will provide baseline (pre-vaccine introduction) data. To assess vaccine impact, we will continue the GEMS case-control methodology. It is estimated that enrollment into the current proposal will begin approximately 24 months after GEMS activities were completed. While we recognize that the gap in incidence measurement is not optimal, there are several reasons to be assured that changes in rotavirus incidence observed in this study can be attributed to rotavirus vaccine introduction. For one, it is well-documented that the incidence of rotavirus-specific medically attended diarrhea is inversely correlated with vaccine coverage, which will be measured throughout the study. Secondly, it is unlikely that potential confounding factors such as economic development will affect rotavirus incidence because rotavirus is generally insensitive to socioeconomic status or changes in water and sanitation, nutritional status, and other factors that might be influenced by economic development. Nonetheless, we will capture other interventions and events that will inform us about factors that might be impacting the incidence of disease. For example, the case report forms used in GEMS (pre-introduction) and that will continue to be used post-introduction assess whether cases and controls have access to improved water and sanitation and data are captured to allow us to calculate a wealth index for each household in the study. Data will be collected at each site on an ongoing basis on local monthly rainfall and peak temperature, which will allow us to examine whether climate-related changes are occurring.

11.3. Impact of vaccine introduction on adverse clinical outcomes following an episode of MSD

Outcomes. All cases and controls enrolled in the case-control study (described above) will participate in the assessment of the following adverse clinical consequences during the ~60 day follow-up period:

- Persistent diarrhea (lasting 14 days or longer): site-stratum-specific difference in the frequency of persistent diarrhea following an episode of MSD pre- and post-vaccine introduction
- Growth faltering, defined as a negative ΔHAZ, where ΔHAZ is the change between enrollment and follow-up in the height-for-age z-score (HAZ)
- A greater degree of stunting, where stunting is a categorical variable defined as HAZ <-1 and graded as mild (-2 $\leq$HAZ <-1), moderate (-3 $\leq$ HAZ <-2), or severe (HAZ <-3).
- Mortality

Analysis. To identify children with persistent diarrhea, their parent/guardian will complete a Memory Aid[24] on which they will indicate whether or not the child had diarrhea for each of the 14 days after study enrollment (**Figure 2**). The proportion of cases that develops persistent diarrhea and the incidence of MSD-associated persistent diarrhea in the DSS population at each site can be compared before and after vaccine introduction. To assess growth faltering and mortality, a single follow-up visit will be made ~60 days after enrollment (acceptable range 50-90 days) to determine vital status and to repeat anthropometric measurements. Vital status will be determined during the enrollment visit and follow-up visit. The proportion of cases that die or become stunted after and episode of MSD and the incidence of MSD-associated mortality or stunting in the DSS population at each site can be compared before and after vaccine introduction.

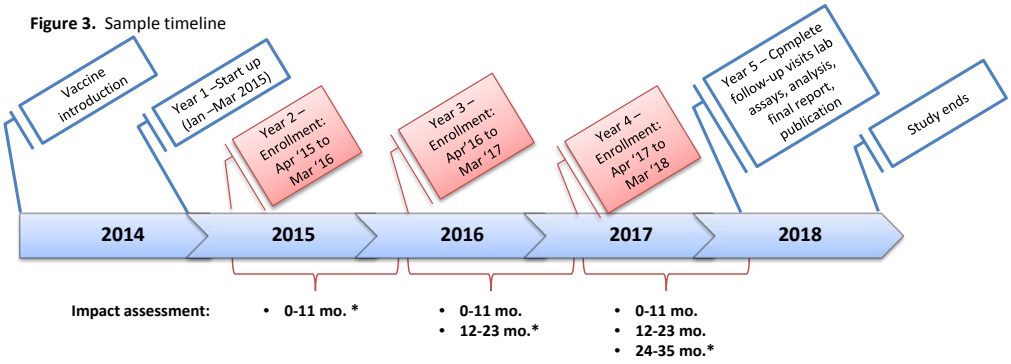
Multivariable analyses will be undertaken to assess pathogens and risk factors associated with each of these outcomes. Verbal autopsies will be performed for all deaths among DSS children aged 0-59 months detected by village informants, study staff, and DSS team. The cause of death will be determined by adjudication per WHO standards.[33] Two physicians will review the outcome of the VA interview and formulate a cause of death. If there is a discrepancy, a third physician will arbitrate the result. The opinion of each physician will be recorded separately in the database, along with the consensus finding. Computer algorithms may also be used to perform this analysis, such as inter-VA4.[33]

11.4. Impact of vaccine introduction on rotavirus-associated MSD

Outcomes. The main outcome will be post-vaccination incidence of rotavirus divided by pre-vaccination incidence of rotavirus, expressed as an incidence rate ratio (IRR). The IRR of MSD will also be determined.

Analysis. Cases for assessment of rotavirus-associated MSD impact will be those enrolled in the diarrhea etiology study, as described above, who are found to be rotavirus-positive. Direct impact would be expected to be seen among infants <1 year of age in the first year of the study, in those <2 years of age in the 2nd year, and <3 years of age in the 3rd year. Reductions in children too young or too old to be vaccinated would be indicative of indirect (herd) protection. Using time series models, with the 4 years of data that has already been collected as part of GEMS, we can forecast the incidence of overall and rotavirus-positive MSD cases that would be expected if no intervention was introduced, the forecast can be compared against the incidence that is actually observed after vaccine roll-out to calculate vaccine impact, using time series models, (generalized linear models of the Poisson family). Monthly incidence of rotavirus MSD "expected" to occur in the absence of a rotavirus vaccination program will be determined by fitting the model to the pre-vaccine (GEMS) data. The model will be adjusted for seasonality by including calendar month and for secular trends by including calendar year. The absolute number of rotavirus MSD cases observed in the vaccine era will be compared with those expected in the absence of vaccination, as computed by the model, to assess the potential impact of vaccination. The rate ratio for rotavirus MSD cases compared with pre-vaccine years will be calculated with the inclusion of an indicator variable for the period after rotavirus vaccine introduction controlling for seasonal and population trends. These statistics can be calculated for a variety of outcomes, including diarrhea, MSD and rotavirus-associated MSD.

Figure 3. Sample timeline



*** During this year, we will begin to assess impact in the under-1 year population; however, the full impact will not be observed until expected levels of coverage (≥ 70%) has been achieved. This will likely occur by 2016; therefore, the full impact can be assessed for 2 years in the <1 year population, and for 1 year in the <2 year population.**

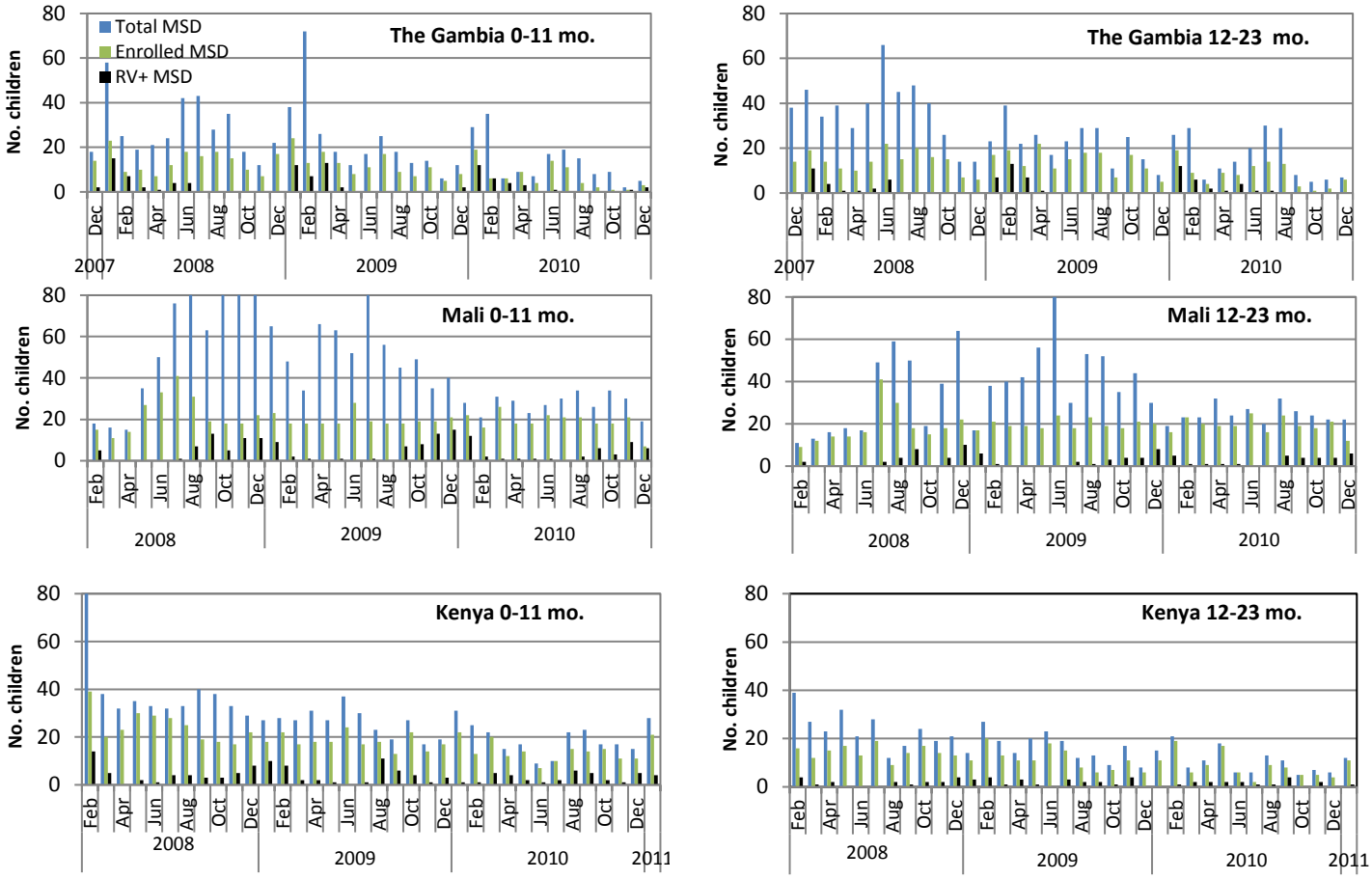
Several years of observation are necessary to observe the impact of vaccine introduction. In The Gambia and Mali, for example, we will begin enrolling subjects in the second quarter of 2015, approximately 15 months after vaccine was introduced. It is likely that coverage will have been suboptimal while the vaccine was rolled-out in 2014, so the full impact cannot be assessed in the infant population until 2015. Nonetheless, three years of enrollment will allow us to observe the impact in the under-one population for 3 years, and in the under-two population for 2 years (**Figure 3**). Multiple years of observation will also allow us to distinguish more precisely changes that occur as a result of vaccine impact from seasonal and temporal variations.

11.5. Effectiveness of rotavirus vaccine

Outcomes. Vaccine effectiveness is calculated as (1 – Odds Ratio)*100, where the odds ratio is the adjusted odds ratio for the rotavirus immunization rate among case-patients compared with controls.

Analysis. Cases will be children participating in the diarrhea etiology study described above who meet the following criteria: i) age-eligible for vaccination; ii) vaccination status can be verified with a medical record or vaccination card; iii) stool sample positive for rotavirus. We will use a sampling scheme (VIDA-plus) in which additional children with MSD will be enrolled and tested only for rotavirus to ensure sufficient power to assess vaccine effectiveness, as described below. We have observed in the GEMS that there are ample additional MSD cases during rotavirus season to expand recruitment numbers. In Kenya, there are no seasonal peaks, so additional enrollments could be continued year round (**Figure 4**).

Figure 4. Total MSD, enrolled MSD, and rotavirus-positive MSD, by month in GEMS



For each case of rotavirus-positive MSD, two types of controls will be selected from among those already enrolled in the case-control study; specific criteria for matching by age and time of presentation will be detailed in the clinical protocol:

1. Rotavirus test-negative control group: The first control group will consist of rotavirus test-negative MSD cases who: i) are age-eligible for vaccination (i.e., born on or after 8 weeks before vaccine introduction and ≥8 weeks old; and ii) have a vaccination status that can be verified with a medical record, vaccination card, or computerized database. These controls will allow us to determine the background frequency of vaccination among infants who have similar health care seeking behavior for diarrheal diseases.

2. "Healthy" (diarrhea-free) community control group: The second group of controls will be selected from the community controls enrolled in the diarrhea etiology study, based on the following criteria: i) no diarrhea in the previous 7 days; ii) age-eligible for vaccination (i.e., born on or after 8 weeks before vaccine introduction and is ≥8 weeks old; and iii) vaccination status can be verified with a medical record, vaccination card, or computerized database. These controls will allow us to determine the background frequency of vaccination in infants who are free of rotavirus MSD.

It has been demonstrated in Kenya that children hospitalized for acute diarrhea who test negative for rotavirus have more similar sociodemographic variables to rotavirus-positive cases, in addition to the obvious similarity in healthcare-seeking behavior.[34] Therefore, we expect that this group will be more suitable as the primary comparator in the event of discordance. However, we also intend to investigate several issues to assist in interpretation of the findings from the two control groups. For one, we will

compare characteristics such as sociodemographic features and health seeking behavior among rotavirus positive MSD cases and the two control groups to assess which control group appears best matched to the cases. In addition, as a measure of bias, we will determine the effectiveness of pentavalent (diphtheria, pertussis, tetanus, *H. influenzae* type b and hepatitis b) vaccine for prevention of rotavirus-associated MSD. Separate analyses comparing cases to community controls and cases to test-negative MSD controls will be performed. After adjusting for potential confounders, any demonstration of vaccine effectiveness could be ascribed to residual selection bias.[34]

NOTE ON CASE ASCERTAINMENT WITH PARTICULAR REFERENCE TO MALI. We have undertaken several steps to overcome methodological issues that we believe obscured case detection in the first year of the Malian RotaTeq trial. For one, because traditional healers are often the main source of health care for MSD,[24,35] surveillance for diarrheal disease at health centers failed to capture many cases. After we initiated community sensitization and established working relationships with the traditional healers serving our DSS in year 2 of the trial, and intensified these efforts during GEMS,[35] our ability to enroll MSD cases improved substantially; notably, Mali had the highest enrollment among the GEMS sites. Another consideration is that the Vesikari scale (used to classify diarrhea by severity in rotavirus vaccine trials) may not be optimal for identifying severe rotavirus diarrhea in low resource communities, and the inclusion of milder cases diminishes measured efficacy. Limitations of the Vesikari scale in this setting include: i) some parameters are irrelevant, e.g., children are rarely hospitalized for diarrhea, even if dehydrated, and temperatures are not measured to diagnose fever; and ii) documentation of signs and symptoms to score severity is based almost entirely on parental report, which is subject to recall and ascertainment bias,[36] whereas the tool was designed to use written documentation with a home diary and inpatient observations. Nonetheless, we will collect data in such a way that analysis can be performed using the Vesikari scale and several available severity scoring systems.[37,38]

We will conduct a nested case-control study to measure vaccine effectiveness for the following reasons: 1) these studies will be initiated ~12 months or less from the onset of vaccine introduction, before full series coverage rates are high enough to detect a measurable impact by monitoring disease incidence alone; and 2) given some natural secular variation in disease incidence, it would be prudent to have an effectiveness evaluation to complement the disease trend analysis. We will compare the proportion of overall or rotavirus-positive MSD cases that were vaccinated against rotavirus to the proportion of rotavirus-negative diarrhea and community controls that were vaccinated. Coverage rates will be low during year 1, so the study will continue for 3 years to best capture the impact of high coverage rates among children aged 0-24 months. Additional information that can be gleaned from the case-control study includes: i) effectiveness of a complete and a partial vaccine series; and ii) duration of protection. Potential risk factors for vaccine failure will be examined, including nutritional status and concomitant infection with enteropathogens that may modulate the immune system or the expression of diarrheal symptoms, such as *Giardia* (shown to "protect" against MSD in GEMS, i.e., more common among controls, and so could in theory interfere with vaccine take), soil helminths, and *H. pylori* (The latter two agents will be detected by TaqMan).

11.6. Secretor and Lewis phenotype as risk factors for vaccine failure

Primary analysis will be conducted among children confirmed to have a full course of rotavirus immunizations.

Outcomes: Among vaccinated infants, the association between secretor status (secretor, non-secretor) or Lewis phenotype (Lewis positive, Lewis negative) and risk of rotavirus vaccine failure (rotavirus-positive diarrhea) will be evaluated. The relative odds of each phenotype (secretor and Lewis) among rotavirus-

positive diarrhea cases and matched controls will be compared. Stratification by infecting rotavirus serotype will be conducted in all analyses.

Analysis: Eligible rotavirus-positive cases and healthy community matched controls will be used in this analysis. Conditional logistic regression will be used to calculate odds ratios. Unconditional logistic regression, adjusting for matching factors (age, sex, community), will be used if there is a low number of discordant pairs. Secondary analyses include stratification of primary analyses by infecting rotavirus serotype (P[8], P[6], and P[4]) and assessing the relationship among children with at least 2 doses of vaccine. Sensitivity analyses include adjusting primary analyses for precision variables: wealth index (proxy for socioeconomic status), anthropometric measures, and oral polio vaccine administration

12. Statistical power and sample size justification

12.1. Sample size considerations for HUCS

The primary endpoint of the survey at a participating site is the proportion, r, of children 0-59 months of age with MSD during the previous two weeks who received care at a sentinel health center associated with the site within 7 days of the onset of the illness.

In GEMS, surveys similar to the HUCS were conducted periodically, and r was estimated by a survival analysis using data from all the survey rounds combined. We plan to use the same procedure, combining data for 6 surveys per site, in VIDA. Assuming the same proportions of MSD in the surveys and the same values of r in VIDA as in GEMS, the approximate sample sizes and standard errors (SE's) of the r estimates in VIDA are given in the table below. The projected SE's are slightly smaller than the calculated SE's in GEMS for The Gambia and Kenya, and substantially smaller for Mali.

Sample sizes over all HUCS rounds and projected SE of r

		GEMS			VIDA	
SITE	Age (mo)	Total sample	r	SE of r	Total sample	SE of r
The Gambia	0-11	2051	0.347	0.034	2700	0.030
The Gambia	12-23	2057	0.258	0.028	2700	0.024
The Gambia	24-59	2196	0.223	0.035	2700	0.032
Mali	0-11	1021	0.221	0.037	2700	0.023
Mali	12-23	987	0.170	0.045	2700	0.027
Mali	24-59	1008	0.094	0.052	2700	0.032
Kenya	0-11	18,817	0.205	0.014	22,600	0.013
Kenya	12-23	19,450	0.190	0.012	23,300	0.011
Kenya	24-59	58,225	0.157	0.011	69,900	0.010

12.2. Case-control study of diarrhea etiology (Table 4)

Table 4 provides site-age-stratum-etiology-specific minimal detectable absolute difference in the proportion estimates and the minimal detectable odds ratios pre- and post-vaccine introduction with 80%

power (two-sided α= 0.05) based on the pre-vaccine attributable fractions and a sample size at each site of ~600 analyzable cases per stratum per year. To compensate for dropout, migration and other losses to follow-up of up to 10%, we planned to enroll a total of 660 cases per stratum per site to achieve the desired number of analyzable cases post-vaccine introduction.

Table 4. Minimal detectable absolute difference in attributable fraction (AF) and minimal detectable odds ratio (OR) pre- and post-vaccine introduction by etiology, age group, and site

	0-11 months			12-23 months			24-59 months		
	Pre-intro-duction AF	Minimal detectable absolute difference	Minimal detectable OR	Pre-intro-duction AF	Minimal detectable absolute difference	Minimal detectable OR	Pre-intro-duction AF	Minimal detectable absolute difference	Minimal detectable OR
Basse									
Rotavirus	23.5	7.2	1.45	17.0	6.5	1.50	12.1	5.8	1.58
Cryptosporidium	11.7	5.7	1.59	7.7	4.9	1.73	--	--	--
ST-ETEC	4.9	4.2	1.93	8.0	5.0	1.71	9.3	5.3	1.66
Shigella	4.0	3.9	2.04	12.8	5.9	1.57	12.6	5.9	1.57
Bamako									
Rotavirus	21.7	7.1	1.46	11.8	5.8	1.59	3.0	3.5	2.22
Cryptosporidium	14.0	6.1	1.55	4.7	4.1	1.95	--	--	--
ST-ETEC	3.6	3.7	2.10	2.3	3.2	2.43	--	--	--
Shigella	--	--	--	2.4	3.2	2.40	2.0	3.0	2.56
Siaya County									
Rotavirus	19.7	6.9	1.48	13.3	6.0	1.56	3.5	3.7	2.12
Cryptosporidium	9.0	5.2	1.67	8.9	5.2	1.68	2.5	3.3	2.36
ST-ETEC	7.0	4.8	1.77	6.9	4.7	1.77	4.9	4.2	1.93
Shigella	4.5	4.0	1.97	4.6	4.1	1.96	9.6	5.3	1.65

12.3. Sample size for vaccine impact on moderate-to-severe rotavirus diarrhea (Table 5)

In GEMS, the incidence of rotavirus per 100 child-years of observation (95% CI) during the first year of life was estimated to be 3.2 (1.7-4.6), 8.4 (3.5-13.3), and 10.1 (5.4-14.8), in Basse (birth cohort 10,400), Bamako (birth cohort 7,811), and Siaya County (birth cohort 7,174), respectively. Given these birth cohort estimates, and assuming 70% vaccine coverage, the detectable change in incidence of rotavirus MSD among children aged 0-11 months, after one birth cohort is vaccinated, will be 30% or greater in Basse and Bamako, and 20% or greater in Siaya County. At the end of the 3 year study, smaller changes with lower vaccine coverage will be detected with 80% power and 2-sided α= 0.05 (**Table 5**). The power to detect changes in overall MSD incidence would be much higher, as the baseline rates are considerably higher (Basse 134/1000 child-years, Bamako 387/1000 child-years, and Siaya County 513/1000 child-years).

Table 5. Required child-years of observation pre- or post-rotavirus vaccine introduction to detect a given change in under-one rotavirus MSD rate by expected vaccine coverage

Expected Change in <1 yr old Rotavirus MSD Rate (n=birth cohort)	Expected Vaccine Coverage: 40%	50%	60%	70%	80%	90%
Basse (n=10,400)						
20%	71,322	45,185	31,058	22,583	17,110	13,376
30%	31,058	19,570	13,376	9,670	7,283	5,659
40%	17,110	10,719	7,283	5,232	3,915	3,022

50%	10,719	6,675	4,506	3,216	2,389	1,830
60%	7,283	4,506	3,022	2,141	1,578	1,199
Bamako (n=7,811)						
20%	25,770	16,336	11,235	8,174	6,196	4,847
30%	11,235	7,085	4,794	3,507	2,643	2,056
40%	6,196	3,886	2,643	1,901	1,424	1,100
50%	3,886	2,423	1,638	1,171	871	668
60%	2,643	1,638	1,100	781	576	438
Siaya County (n=7,174)						
20%	21,052	13,348	9,182	6,681	5,066	3,963
30%	9,182	5,792	3,963	2,868	2,163	1,682
40%	5,066	3,178	2,163	1,556	1,166	901
50%	3,178	1,983	1,341	959	714	548
60%	2,163	1,341	901	640	473	360

12.4. Sample size for vaccine impact on adverse clinical outcomes following an episode of MSD

12.4.1. Growth faltering

In GEMS, we observed a significant association between MSD and linear growth faltering within the 60-day follow-up period. At enrollment, 50.7% of infants had no stunting, while mild (height for age z score [HAZ] $\leq$-1 and $\geq$2), moderate (HAZ <-2 and $\geq$-3), or severe (HAZ <-3) stunting was present in 30.8%, 12.8%, and 5.8% of infants at follow-up, respectively. At follow-up, 41.5% of infants no stunting, while mild, moderate, or severe stunting was present in 32.1%, 18.1%, and 8.4% of infants at follow-up, respectively. Given a sample size at each site of ~600 analyzable cases pre- and post-vaccine introduction, **Table 6** provides site-age stratum-specific minimal detectable absolute difference in the proportion of children that develop stunting (either mild, moderate or severe) or become more stunted during the 60 day follow-up period pre- and post-vaccine introduction and the minimal detectable odds ratios with 80% power (two-sided α= 0.05) given the proportion of children that faltered in growth during the pre-vaccine follow-up period. A change in stunting of at least 30% could be detected in infants and in toddlers at Basse and Siaya County.

Table 6. Minimal detectable absolute difference in the change in the prevalence of stunting after MSD and minimal detectable odds ratio (OR) pre- and post-vaccine introduction by age group and site

	% Pre-introduction that developed stunting or became more stunted after an MSD episode*	Minimal detectable absolute difference	Minimal detectable OR
Basse			
0-11 months	25.1	7.4	1.44
12-23 months	28.8	7.6	1.42
24-59 months	12.0	5.8	1.59
Bamako			
0-11 months	19.1	6.8	1.48
12-23 months	10.4	5.5	1.63
24-59 months	4.7	4.1	1.95
Siaya County			
0-11 months	24.9	7.3	1.44
12-23 months	23.7	7.2	1.44
24-59 months	16.0	6.4	1.52

*Calculated as the proportion of children who had a difference (60-d follow-up minus enrollment) in stunting score $\geq$1, scored as 0=none, 1=mild (height for age z score [HAZ] $\leq$-1 and $\geq$2), 2=moderate HAZ <-2 and $\geq$-3), 3=severe (HAZ <-3).

12.4.2. Persistent Diarrhea (Table 7)

In GEMS, persistent diarrhea occurred among 33/748 cases of MSD from Basse (4.4%), 125/1738 MSD cases in Bamako (7.2%), and 184/1336 MSD cases from Siaya County (13.8%), when the analysis was limited to children whose caretakers completed the 14-day memory aid. Given a sample size at each site of ~600 analyzable cases pre- and post-vaccine introduction, **Table 7** provides site-stratum-specific minimal detectable absolute difference in the frequency of persistent diarrhea following an episode of MSD pre- and post-vaccine introduction and the minimal detectable odds ratios with 80% power (two-sided α= 0.05) given the pre-vaccine frequency of persistent diarrhea following an episode of MSD. As shown in **Table 7**, it is likely that only in Kenya is such an analysis feasible, and even in Kenya only large differences (~42-45%) would be detectable.

Table 7. Minimal detectable absolute difference in the prevalence of persistent diarrhea and minimal detectable odds ratio (OR) pre- and post-vaccine introduction by age group and site

	Pre-introduction prevalence of persistent diarrhea	**Minimal detectable absolute difference**	**Minimal detectable OR**
Basse			
0-11 months	3.8	3.8	2.07
12-23 months	6.2	4.5	1.82
24-59 months	0.8	2.3	3.85
Bamako			
0-11 months	6.4	4.6	1.80
12-23 months	7.2	4.8	1.75
24-59 months	8.1	5.0	1.71

Siaya County			
0-11 months	15.0	6.3	1.53
12-23 months	14.2	6.2	1.54
24-59 months	13.8	6.1	1.55

12.4.3. Sample size for vaccine impact on diarrhea-associated mortality (Table 8)

Since verbal autopsy data are not yet available from the GEMS data, we used the average all-cause diarrhea mortality for children 0-11 months of age in Basse and Siaya County from 2007-2012 (5.835 diarrhea deaths per 1,000 population aged 0-11 months) as determined by the interVA4 system to estimate the sample size need to reduce (one-sided α= 0.05) all-cause diarrhea mortality among children 0-11 months of age. Table 8 provides the number of child-years of observation among children 0-11 months of age for a range of reductions in under-1 diarrhea mortality rate and vaccine coverage in the under 1 population with 80% power. Given a combined under-1 population of ~25,000 at the three sites followed for 3 years (75,000 person-years <1 year of age), only very large reductions (≥30%) with high vaccine coverage (≥60%) in the under 1 population would be detectable. There would not be sufficient power to detect site-specific reduction in all-cause diarrhea mortality in children <1 year of age. However, statistical power would increase if observations were extended for additional years.

12.5. Sample size for vaccine effectiveness case-control evaluation

In GEMS, among infants 0-11 and 12-23 months of age, respectively, the mean annual number of rotavirus-associated MSD cases was: The Gambia (67 and 53), Mali (123 and 53), and Kenya (51 and 30); and the mean annual number of MSD cases was: The Gambia (260 and 296), Mali (550 and 397), and Kenya (355 and 196). Following vaccine introduction, the number of rotavirus cases is expected to be reduced by 50% resulting in 33 and 27 cases per year in The Gambia, 61 and 26 in Mali, and 25 and 15 in Kenya for children 0-11 months and 12-23 months respectively. However, continuous sampling of all MSD cases during the rotavirus seasons will increase rotavirus case enrollment numbers and help to ensure a sufficient number of cases. Sample size requirements will be adjusted for non-inclusion of ~15% of subjects.

Table 8. Required person-years of observation pre- or post-rotavirus vaccine introduction to detect a given reduction in under-one rotavirus MSD mortality rate by expected vaccine coverage

Expected Reduction in <1 Diarrhea Mortality Rate	Expected Vaccine Coverage:						
	30%	40%	50%	60%	70%	80%	90%
10%	2,485,776	1,399,009	892,499	615,857	449,742	342,327	268,936
20%	615,857	342,651	217,438	149,228	108,377	82,031	64,218
30%	268,936	149,132	93,869	64,218	46,363	34,937	27,116
40%	149,228	82,111	51,414	32,008	25,064	18,887	14,457

12.5.1. Analysis Using Test Negative Controls (Table 9).

The sample size is calculated to achieve 80% power at the 5% significance level using the following parameters and assumptions:

- Detect an odds ratio of at least 0.5 or vaccine effectiveness of 50%

- Case-patient to control ratio of 1:3 to 1:6 or 14% to 25% of enrolled patients testing positive for rotavirus depending on site and age strata (see below for rotavirus detection rates by site and age)
- Vaccine coverage of 70% for a full series, for age-eligible controls.

Using these parameters and assumptions, approximately 80-92 case-patients and 276-480 test-negative controls are needed to demonstrate a vaccine effectiveness of ≥50% with 70% vaccine coverage depending on the rotavirus detection rate at the site. However, given the uncertainty of the vaccine uptake and coverage in the population and the field effectiveness of the vaccine, a wide range of scenarios were examined (**Table 9**).

Table 9. Required number of cases* to detect a given vaccine effectiveness by expected vaccine coverage

Expected Vaccine Effectiveness	Expected Vaccine Coverage: 40%	50%	60%	70%	80%	90%
1:3 case to control ratio* (25% rotavirus detection rate)						
70%	40	34	30	30	33	50
60%	63	55	51	52	60	94
50%	104	92	88	92	111	179
40%	183	165	162	173	213	354
30%	363	334	333	364	457	776
1:4 case to control ratio (20% rotavirus detection rate)						
70%	38	32	28	28	31	45
60%	59	51	48	48	56	87
50%	97	86	82	86	103	165
40%	172	155	151	162	199	329
30%	340	313	312	340	427	723
1:6 case to control ratio (14% rotavirus detection rate)						
70%	35	29	26	26	28	41
60%	56	48	44	45	51	79
50%	91	80	77	80	95	152
40%	160	145	141	150	184	304
30%	318	292	291	317	396	670

*To determine the number of controls, multiply the number of cases by 3 (for 3:1 control to case ratio)

12.5.2. Analysis Using Matched Community Controls (Table 10).

The sample size is calculated to achieve 80% power at the 5% significance level using the following parameters and assumptions:

- Detect an odds ratio of at least 0.5 or vaccine effectiveness of 50%
- Case-patient to control ratio of 1:1
- Vaccine coverage of 70% for a full series, for age-eligible controls.

Using these parameters and assumptions, approximately 141 case-patients and 141 matched community controls are needed to demonstrate a vaccine effectiveness of ≥50% with 70% vaccine coverage. However, given the uncertainty of the vaccine uptake and coverage in the population and the field effectiveness of

the vaccine, a wide range of scenarios were examined (**Table 10**). Case recruitment will continue irrespective of numbers achieved throughout the duration of the project.

Table 10. Required sample size of cases (or controls) to detect a given vaccine effectiveness by expected vaccine coverage given 1:1 control to case ratio

Expected Vaccine Effectiveness	Expected Vaccine Coverage					
	40%	50%	60%	70%	80%	90%
70%	58	50	46	46	53	81
60%	92	81	77	80	95	151
50%	152	137	133	141	172	283
40%	270	247	244	264	329	554
30%	539	499	502	553	700	1198

12.6. Sample size for secretor and Lewis phenotype as risk factors for vaccine failure

The main exposures in both aims are *FUT*2 and *FUT*3 phenotypes. Previous studies have found *FUT*2 non-secretor individuals to be 20% of an African population and *FUT*3 Lewis-negative individuals to be 35% of an African population.[9] To observe an odds ratio of 1.5-2 we aim to enroll at least 160 cases and matched controls (1:1 – 1:3, depending on the proportion of cases from VIDA and VIDA+). The analysis assumes a type I error α of 0.05 and power of 0.8.

13. Risks and Benefits

13.1. Potential risks

The risks to participating are minimal, and could include the following:

1. Rectal swabs may cause some temporary discomfort but are not known to cause any injury. Clinicians will be trained in the collection of rectal swabs.
2. Saliva collection is non-invasive, but may cause temporary discomfort.
3. There is a potential risk for breach of confidentiality. To minimize the risk, CRFs will be stored in locked file cabinets at the sites. The files can be accessed only by study personnel. Forms that contain personal identifiers (the child's name, address, and other identifiers that will permit study personnel to perform a home visit 60 days after enrollment) will be maintained at the sites under secure conditions with limited access and not transmitted off-site to the central database. The remaining CRFs will be labeled only with the child's study number. The data on the Census ID Log will be sent to the DCC. This log may include information from the census such as the child's census ID number, village name, census cluster number, compound number, household number, and global positioning system (GPS) coordinates. Although no names will be recorded, this information could potentially be linked to a child's identity. Therefore, as a precaution to maintain confidentiality, the log will be sent to the DCC via email separate from the clinical and epidemiologic information that is collected and will be stored at the DCC in a secure location, separate from the case report forms.
4. Collection of information for research purposes from parents of children who are ill could contribute to emotional upset. Study personnel will be trained to collect information about these illnesses in a respectful and sensitive fashion.

13.2. Potential benefits

There is no benefit that can be guaranteed to participants. The results of the cultures for bacterial pathogens will be provided to the health care providers in a timely fashion. At some sites, this information is not normally available. An etiologic diagnosis may lead to more effective treatment of a child's illness. It is hoped that this study will guide the prioritization and development of strategies that will effectively prevent diarrheal illnesses and deaths in young children. In Kenya, caregivers of children enrolled in the study may be provided with transportation from the health center back to their home to mitigate inconvenience caused by study activities and to ensure that VIDA's inducements are aligned with those of similar studies at the site.

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