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Characteristics and Etiology of Moderate-to-Severe Diarrhea of Acute, Prolonged Acute, and Persistent Duration among Children Less than 5 Years Old in Rural Western Kenya, 2008-2010

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Abstract

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Characteristics and Etiology of Moderate-To-Severe Diarrhea of Acute, Prolonged Acute, and Persistent Duration among Children Less Than 5 Years Old in Rural Western Kenya, 2008-2010

(Under the direction of Dr. Richard Rothenberg, MD, MHP, FACP and Dr. Eric Mintz, MD, MPH.)

Worldwide, diarrheal disease is the second leading cause of death in children under 5 years old. Data on diarrhea of extended duration is limited. We described the characteristics associated with acute, prolonged acute and persistent diarrhea in Kenyan children less than 5 years of age participating in the Global Enterics Multicenter Study. Children presenting at a clinic were enrolled if they met the case definition for acute moderate-to-severe diarrhea defined as ≥3 loose stools in the last 24 hrs, within 7 days of illness onset, with ≥1 of the following: sunken eyes, skin tenting, dysentery, IV rehydration, or hospitalization. To determine diarrhea duration, the child's caretaker was asked to recall the number of days the child had diarrhea in the 7 days pre-enrollment, and to record each day of diarrhea post-enrollment on a form for 14 days. Stool specimens were collected at enrollment, and the post-enrollment form was collected during a home visit. We defined acute diarrhea (AD) as ≤6 days duration, prolonged acute diarrhea (ProD) as 7-13 days, and persistent diarrhea (PD) as ≥14 days. From January 31, 2008 to January 24, 2010, 557 children with acute moderate-to-severe diarrhea were enrolled. Using the Wilcoxon rank-sum test, Kruskal-Wallis test, and Cox Proportional Hazards Model we examined the relationship between the duration of diarrhea by gender, age, and various etiologic agents. We found no association between gender and the duration of diarrhea. Age was associated with diarrhea of extended duration; children less than or equal to 11 months of age were 1.3 times more likely to experience diarrhea of longer duration than their counterparts. We found *Cryptosporidium* to be more associated with ProAD and PD. Children infected with *Cryptosporidium* were 1.5 to 1.7 times more likely to have diarrhea with a longer duration than their counterparts. Based on these results, interventions related to diarrhea and diarrhea of extended duration should focus more closely on young children, especially children less than 24 months of age.

Characteristics and Etiology of Moderate-To-Severe Diarrhea of Acute, Prolonged Acute, and Persistent Duration among Children Less Than 5 Years Old in Rural Western Kenya, 2008-2010

By Katharine A. Schilling

Bachelor of Science in Health Education and Health Promotion, minor Nutrition from Central Michigan University, December 2007

A Thesis Submitted to the Graduate Faculty of Georgia State University in Partial Fulfillment of the Requirements for the Degree

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Characteristics and Etiology of Moderate-To-Severe Diarrhea of Acute, Prolonged Acute, and Persistent Duration among Children Less Than 5 Years Old in Rural Western Kenya, 2008-2010

By

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CHAPTER I

INTRODUCTION

1.1 Background

Worldwide, diarrheal disease is the second leading cause of death in children under 5 years old.[1] According to the World Health Organization (WHO), each year approximately 1.5 million children die as a result of diarrheal diseases.[2] Bacterial, viral, and parasitic agents ingested through contaminated drinking water or food can result in diarrhea.[2] Enormous numbers of people lack access to improved water and sanitation, 884 million and 2.6 billion, respectively.[3]

In Kenya, diarrhea is a leading cause of morbidity. According to the Demographic and Health Survey (DHS) conducted in 2008-2009, diarrhea accounted for 17% of childhood illnesses.[4] Younger children are often at greater risk. In fact, 30% of children with diarrhea were between 6 and 11 months of age.[4] Many people living in Kenya do not have access to safe water or adequate sanitation, especially people living in rural areas.[4] Roughly, 37% of households do not have access to an improved drinking water source and more than 75% of households do not have access to an improved sanitation facility.[4]

Recently, rates of acute diarrhea have declined, but a larger percentage of the burden of diarrhea morbidity is attributed to persistent diarrhea.[5] Diarrhea of extended duration has been associated with a number of adverse health consequences including delays in growth,[6] nutritional deficiencies,[7] and decreased cognitive function over time.[7,8] Importantly, children presenting with PD have experienced higher rates of death as compared to children experiencing AD.[9] Risk

factors influencing the duration of diarrhea include malnutrition,[10-12] mother's education level,[13,14] previous illness,[7,10] time of weaning,[14] bloody diarrhea,[15,16] and the age of a child when they first experience diarrhea.[13]

In the past 10 years, few studies relating to the duration of diarrhea have been published, although diarrhea of extended duration continues to be a significant health problem.[5] Due to this and to limitations in the current literature, more research should focus on diarrhea of extended duration.[17]

1.2 Purpose of Study

The purpose of this study is to describe the characteristics associated with acute, prolonged acute and persistent diarrhea in Kenyan children less than 5 years of age participating in the Global Enterics Multicenter Study (GEMS).

For the purposes of this study, diarrhea was defined as 3 or more loose stools in a 24 hour period as suggested by the WHO.[2] The duration of diarrhea was defined by the following three categories: acute diarrhea (AD), diarrhea lasting between 1 and 6 days; prolonged acute diarrhea (ProAD), diarrhea lasting 7 to 13 days; and lastly, persistent diarrhea (PD), diarrhea lasting 14 or more days.

1.3 Research Questions

This thesis will address the following questions: 1) Is age associated with a greater likelihood of ProAD or PD compared to AD? 2) Is gender associated with a greater likelihood of ProAD or PD compared to AD? 3) Are any etiologic agents associated with a greater likelihood of ProAD or PD compared to AD?

CHAPTER II

REVIEW OF THE LITERATURE

The purpose of this study is to examine characteristics associated with the duration of diarrhea in Kenyan children less than 5 years old. This literature review will focus on the following areas: the global burden of disease, factors influencing the duration of diarrhea, and the burden of diarrheal diseases in Kenya.

2.1 Diarrheal diseases

2.1.1 Global burden of diarrheal diseases

Worldwide, diarrheal disease is the second leading cause of death in children under 5 years old.[1] According to the World Health Organization (WHO), each year approximately two billion people become ill with diarrhea, resulting in the death of approximately 1.5 million children.[2] The most severely affected population includes children under 2 years of age.[2] In 2004, 80% of the 1.5 million children that died due to diarrheal illness were less than 2 years old.[2] In 2004, the estimated Disability Adjusted Life Years (DALYs) associated with diarrheal disease was 72.8 million, meaning that 72.8 million years of life were lost due to disability and death associated with diarrheal disease.[18] This figure ranked second behind lower respiratory infections, and represents 4.8% of global DALYs.[18]

2.1.2 Mode of transmission

Diarrheal diseases are most often caused by bacterial, viral, or parasitic agents, spread by way of fecal oral contact.[19] There are additional causes of diarrhea, such as food intolerances,

adverse effects of medications, intestinal diseases, and bowel disorders, but these conditions are beyond the scope of this paper. Commonly, bacterial, viral, and parasitic agents are ingested through contaminated drinking water or food as a result of poor hygiene or inadequate water treatment and sanitation facilities.[2] In fact, it has been estimated that 88% of all diarrheal diseases are caused as a result of contaminated water and inadequate hygiene and sanitation.[20]

Lack of access to safe water and adequate sanitation facilities is a serious problem worldwide, where approximately 884 million people lack access to improved water sources and 2.6 billion people do not have access to improved sanitation facilities.[3] This leads to open defecation and the improper disposal of feces. It is estimated that in developing countries, 25% of people defecate in the open.[1] Improper disposal of a child's feces has a potentially greater consequence since their stool is often more heavily contaminated with pathogens than that of adults.[1]

2.1.3 Clinical manifestations

Generally, diarrhea presents as watery or loose stools, but it can also present as bloody, loose stool, also known as dysentery.[2] Other symptoms may include stomach pain, nausea, vomiting, and fever.[19] If not properly attended to, diarrhea can be particularly problematic because it results in dehydration which can lead to death. Dehydration occurs when the body has inadequate amounts of fluid and electrolytes to function properly.[19] Signs of dehydration may include sunken eyes, abdomen, or cheeks, skin tenting, irritability, inability to produce tears or sweat, dry mouth, and fever.[19]

2.1.4 Prevention and treatment of diarrheal illness

The WHO has identified a number of recommendations for preventing diarrhea in developing countries. These include: improved access to water and sanitation, increased handwashing with soap, promotion of exclusive breastfeeding for the first 6 months of life, improved nutrition to decrease the number of children who are malnourished, increased use of Vitamin A and zinc, and finally, increased rates of immunization against both rotavirus and measles.[1] The WHO treatment recommendations for diarrheal illness include the use of oral rehydration therapy to replenish lost fluids and electrolytes, continued feeding as the child experiences diarrhea, and administration of zinc for 10-14 days (10mg for <6 months and 20mg for those >6 months).[21]

2.2 Duration of diarrheal illness

In the following section, I will discuss the health implications due to diarrhea of extended duration, discuss the definitions associated with length of diarrhea, and identify factors influencing the duration of diarrhea.

2.2.1 Definitions of diarrheal duration

Definitions relating to the duration of diarrhea are often inconsistently used. WHO has clearly defined persistent diarrhea (PD) as diarrhea lasting more than 14 days,[2] but by default then, acute diarrhea (AD) is typically defined as diarrhea lasting less than 14 days. Most recently, Moore and colleagues[14] have identified prolonged acute diarrhea (ProAD) as diarrhea lasting between 7 and 13 days. They found this to be an important durational length as their investigation found it was predictive of future persistent diarrheal episodes and the consequences there of. Based on these results, they have proposed the following definitions be adopted by

WHO: AD defined as diarrhea lasting less than 7 days and ProAD defined as diarrhea lasting between 7 and 13 days. Figure 2.1 lists the definitions of diarrheal duration as described above.

Figure 2.1. Definitions of Diarrheal Duration

Definitions of Diarrheal Duration
Acute Diarrhea (AD) – 1 to 6 days of diarrhea
Prolonged Acute Diarrhea (ProAD) – 7-13 days of diarrhea
Persistent Diarrhea (PD) – 14 or more days of diarrhea

2.2.2 Prolonged acute diarrhea

Bhutta and collegueas,[5] as part of a Persistent Diarrhea working group, have identified focus areas for future work related to diarrhea of extended duration. One suggested research area includes focusing on "prolonged diarrhea" or diarrhea lasting longer than 5 to 7 days, but not exceeding 14 days.[5] To my knowledge, there is only one study focusing specifically on the duration of diarrhea lasting between 7 and 13 days, ProAD. In this study, Moore and colleagues[14] examined data from a prospective cohort study to explain the epidemiology of ProAD in children less than 5 years old living in an urban shantytown in northeastern Brazil. Children were followed from August 1989 to March 2000. The children were routinely assessed for the presence of diarrheal illnesses, their height and weight were recorded on a regular basis, and stool specimens were collected and tested for a wide range of enteric pathogens from both children with or without diarrhea. The study consisted of 414 children experiencing 3,257 episodes of diarrhea, of which 83.6% were classified as AD, 11.7% ProAD, and 4.7% PD. The investigators found that when diarrhea persisted, and moved from "acute" to "prolonged acute"

the risk of experiencing "persistent" diarrhea increased from 4.8% to 29%. Furthermore, infants who experienced ProAD were 2.2 times more likely to experience PD. Risk factors identified by the investigators to be associated with ProAD included the child's age at weaning and the mother's education. In comparison with controls, etiologic agents associated with ProAD included *Cryptosporidium* and *Shigella*. Lastly, they found that poor nutrition both before and after diarrhea were associated with ProAD.

2.2.3 Persistent diarrhea

As stated earlier, PD is defined as diarrhea lasting 14 or more days. As rates of AD have decreased over the years, PD has become a larger percentage of the diarrheal disease burden in children.[5] Of additional importance, higher rates of death have been reported amongst infants presenting with PD as compared to those with AD.[9] Furthermore, PD can lead to a number of health consequences including delays in growth,[6] nutritional deficiencies,[7] and decreased cognitive function over time.[7,8]

In recent years the amount of literature relating to diarrhea of extended duration has decreased, although there is no evidence to suggest that rates of diarrhea of extended duration have declined.[5] Duration of diarrhea has not been studied in a consistent and systematic manner, and a number of limitations exist amongst the literature currently available which will be discussed later.

2.3 Factors influencing the duration of diarrhea

Described below are some factors influencing the duration of diarrhea found in the literature review. Risk factors were limited only to those that were examined in this study.

2.3.1 Age

Age has an important influence on the duration of diarrhea. Most research has consistently found that infants experience PD at higher rates than older children. Several studies have identified that children less than 6 months of age experience higher rates of PD,[11,15,16,22-25] whereas other researchers have found that episodes of PD were highest in children aged 19 to 24 months.[26]

2.3.2 Gender

Most researchers have found no association between gender and diarrhea lasting longer than 14 days.[16,22,23] Interestingly, Cruz and colleagues[22] found that although there was no significant association between males and females 0 to 17 months old experiencing PD, males 18 months and older experienced a larger number of episodes of PD than females. In contrast, among females the frequency of episodes of PD decreased as they grew older. The investigators suggested a few possible explanations for this including differences in immune responses between males and females and the mobility of boys in the community leading to the potential exposure to more pathogens.

2.3.3 Etiologic Agents

This section will describe and review etiologic agents commonly found in children experiencing PD that have been examined as part of this study.

Abba and colleagues[17] recently conducted a comprehensive review of etiologic agents identified in children presenting with PD. They found 19 relevant studies from which they abstracted the data, to determine if across the studies there was an association between different

etiologic agents and PD. The researchers separated the data by WHO defined regions and computed weighted means for each pathogen by diarrhea type (i.e. AD or PD). They found that children experiencing PD had an assortment of pathogens identified in their stool, but for the most part these pathogens were isolated in low frequencies, with the exception of *Escherichia coli (E. coli)* which was isolated from 25% to 33% as compared to less than 10% for any other pathogen. When examining studies with a comparison group, the researchers found that children with PD were more likely to have a pathogen identified than the comparison group, but there was no one particular pathogen that seemed to be more associated with PD. In conclusion, the researchers found there were no etiologic agents that were more likely associated with PD. However, they identified numerous challenges and limitations to this type of analysis including small sample sizes amongst studies, geographic variation, differences in living conditions, dissimilarity in study design and methodologies, length of study period, and the time at which most of these studies were published, which was generally over 10 years ago. All of these factors limit the generalizability and comparability of the research currently available, leading the authors to suggest more research should focus on diarrhea of extended duration. Regardless, this paper is important in that it reveals the variety of pathogens found in children experiencing PD and the limitations to the current literature highlighting the need for future research in this area.

2.3.4 *Campylobacter* spp.

Campylobacter is found commonly in the environment and, children are therefore exposed to the bacteria at a very young age with high infection rates in developing countries.[27] *Campylobacter* infections are most severe in younger children, but as children reach 2 to 3 years of age symptoms are less severe[27,28] and infection is more often asymptomatic.[27,29] However,

children less than 6 months who are breastfed are typically protected against infection.[27] In rural India, *Campylobacter jejuni* was found significantly more often in children with AD than in controls.[30] Most studies focusing on diarrhea of extended duration identified few *Campylobacter* spp. in their study populations, did not report on its impact, or did not test for the organism.[6,16,24,26,31]

2.3.5 Nontyphoidal *Salmonella*

Illness associated with nontyphoidal *Salmonella* is typically self-limited[32] and resolves within 5 to 7 days.[33] Common symptoms include loose, non-bloody stools, stomach pains, nausea, vomiting, fever, and chills.[32] *Salmonella* was identified at high rates in Indian children with PD, but there was no statistically significant association.[16] Most studies examining the duration of diarrhea identified few nontyphoidal *Salmonella* in the study population or did not test for these organisms.[6,16,24,26,31]

2.3.6 *Shigella* spp.

Shigella is found most often in crowded locations with poor sanitary conditions and unsafe water supplies, hence *Shigella* is identified at higher rates in developing countries.[34] It is uncommon in children less than 6 months of age, but is more common among older children, especially those 1 to 5 years old.[34] In developing countries, S*higella* may present as a more severe illness.[34-36] In Bangladesh, when *Shigella* was identified among cases within the first few days of illness, it was borderline significantly associated with PD as compared to controls.[37] Additionally*, Shigella* was isolated at high frequencies in Indian children with PD, but there was no statistically significant association.[16] However, in Brazilian children, *Shigella* was found to have a stronger association with AD.[6]

2.3.7 Enterotoxigenic *Escherichia coli*

Enterotoxigenic *Escherichia coli* (ETEC) is a common enteric pathogen causing diarrheal disease not only in humans, but in domesticated animals as well.[38] Severity of illness associated with ETEC can vary greatly, from mild to profuse watery diarrhea, at times similar to cholera.[38] Additional symptoms less frequently experienced include fever, nausea, chills, vomiting, head and muscle aches, and lack of appetite.[39] Symptoms usually last 3 to 4 days, and typically illness does not exceed 3 weeks.[39] Young children, at the age of weaning are usually more susceptible to develop illness from ETEC.[38] In Brazilian children, ETEC was found to be associated with PD. [6] Whereas, in rural India, ETEC was found significantly more often in children with AD than in controls.[30] ETEC was identified more frequently in children with PD compared to AD in rural Indian, but the association was not statistically significant.[16] In Vietnam, in a sample of children experiencing PD, ETEC was the most frequently identified etiologic agent.[24] And, in another study from Brazil, ETEC was identified more frequently in controls than cases.[26]

2.3.8 Enteropathogenic *Escherichia coli*

An important causative agent of childhood diarrhea in developing countries is enteropathogenic *Escherichia coli* (EPEC), causing approximately 5-10% of diarrheal episodes.[40] Limited knowledge of the clinical presentation of EPEC exists, and can vary depending on population and location.[41] One study found EPEC to be associated with both AD and PD in children living in Brazil.[6]

2.3.9 Enteroaggregative *Escherichia coli*

One distinctive characteristic of enteroaggregative *Escherichia coli* (EAEC) is the extended length of illness associated with infection.[42] Although, the pathogenocity of EAEC has

been questioned because all epidemiologic studies have not associated infection with diarrheal illness.[43] There are a number of possible reasons for this, which are currently being pursued. Some of the reasons may include: the organism is shed in the stool for extended amounts of time after illness has ceased, illness is often asymptomatic, nonpathogenic *E. coli* is included in EAEC as it is currently defined, and lastly, the typical epidemiologic study on diarrhea may miss infections as we do not fully understand the clinical presentation.[43] Studies in northeastern Brazil and rural India, have found EAEC to be associated with PD,[16,30,31] whereas another study in Brazil found EAEC in both cases and controls.[6]

2.3.10 Rotavirus

Rotavirus is a common cause of severe diarrheal illness and sometimes death in young children around the world.[44,45] Infection with rotavirus typically results in watery diarrhea, vomiting, stomach pain, and fever. Duration of illness varies from 3 to 8 days[44] and has been found to be associated with AD, but less frequently with PD.[7,12] In Bangladesh, rotavirus was found to be associated with AD, compared to healthy controls when infection was identified in the first few days of illness.[37] In Lima, Peru, rotavirus was significantly associated with AD as compared to children with PD.[46] In northeastern Brazil, only 3.7% of children presenting to a clinic had rotavirus identified in their stool.[31] In the same city in northeastern Brazil, in a cohort of children, rotavirus was the most frequently identified pathogen among cases with AD or PD and among healthy controls. However, the frequency of identification amongst groups was similar and there was no statistically significant association between any of the pathogens identified in children with AD or PD.[26]

2.3.11 *Giardia lamblia*

Giardia lamblia also referred to as *Giardia intestinalis* is a common intestinal protozoa and may be present in 20-30% of people in the developing world.[47] Symptoms may include a broad spectrum of disease from asymptomatic, self-limiting diarrhea, to chronic diarrhea.[47] Duration of illness can be long, lasting 2 to 6 weeks, of longer in some cases.[48] In children living in northeastern Brazil, *Giardia* was frequently identified in children with PD, but in the association was not statistically significant.[26,31] In other studies, an association was found between *Giardia* and PD in northeastern Brazil and in a New Delhi slum.[6,49] However, *Giardia* was identified infrequently in children living in Bangladesh and there was no association between it and AD or PD.[37]

2.3.12 *Cryptosporidium*

Illness as a result of *Cryptosporidium* usually presents itself as watery diarrhea along with a number of other symptoms including fever, nausea, vomiting, abdominal cramping.[50] Duration of illness can be as short as a few days or as long 4 or more weeks; typically symptoms are present for 1 to 2 weeks.[50] *Cryptosporidium* has been identified as an important pathogen in PD, especially in malnourished children.[7,12] In an urban slum in northeastern Brazil, an association between the presence of *Cryptosporidium* and both AD and PD was found.[6] Additionally, in a similar location in Brazil *Cryptosporidium* was the second leading pathogen identified in children presenting to a clinic with PD.[31] In Bangladeshi children, *Cryptosporidium* was associated with PD compared to children with AD.[37]

2.3.13 Other Risk Factors

Numerous other factors not related to age, gender or etiology have been identified as characteristics influencing the duration of diarrhea. These include malnutrition,[10-12] maternal education level,[13,14] previous illness,[7,10] time of weaning,[14] bloody diarrhea,[15,16] and the age of a child when they first experience diarrhea.[13]

2.4 Diarrheal disease in Kenya

2.4.1 Characteristics of Kenya

Kenya is a country located in sub-Saharan Africa, bordered by Tanzania, Somalia, Uganda, Sudan, and Ethiopia.[51] The population is 39,002,772 with a growth rate of 2.691%.[51] Kenya ranks 147th out of 177 countries on the Human Development Index (HDI),[52] this ranking falls under the classification of "medium human development".[52] The HDI is calculated using three population level measurements which include life expectancy at birth, literacy rates and education levels, and Gross Domestic Product (GDP) per capita.[53]

According to the 2008-2009 Kenya Demographic and Health Survey (DHS)[4] life expectancy at birth was 58.9 years.[4] Infant mortality is 52 deaths per 1,000 live births and under-five mortality is 74 deaths per 1,000 births.[4] Both infant and under-five mortality have declined since the last DHS conducted in 2003.[4] The leading reported causes of morbidity in children under 5 years of age included fever (24%), diarrhea (17%), and acute respiratory infections (8%).

2.4.2 Water sources and sanitation facilities

Approximately, 63% of households in Kenya have access to an improved drinking water source, but there are large differences among urban and rural residents.[4] In urban areas, 89% of households have access to an improved source, whereas only 54% of rural residents have access.[4] A majority (55%) of households' reported not treating their water.[4] In rural areas, households reported not treating their water more frequently than in urban areas, 59% and 43% respectively.[4]

In Kenya, less than 25% of households have access to an improved sanitation facility that is not shared.[4] Again, more households in urban areas have access to private, improved sanitation facilities than those households located in rural areas.[4]

2.4.3 Diarrheal Diseases in Kenya

In the 2008-2009 Kenya DHS 17% of children reported diarrhea in the past 2 weeks and 3% reported bloody diarrhea or dysentery.[4] Diarrhea in this population occurs most frequently in younger children.[4] The highest rates (30%) were reported in children between the ages of 6 and 11 months.[4]

The Kenya DHS found a number of factors that influenced the rates of diarrhea in children under 5 years old. Lower rates of diarrhea were found in children who had access to improved water sources, private, improved latrines, and among children whose mothers had some secondary education. Additionally, diarrheal rates vary by changes in season.[4]

Chapter III

METHODOLOGY

This study will examine data on the duration of diarrhea in Kenyan children enrolled in the Global Enterics Multicenter Study (GEMS) between January 31, 2008 and January 24, 2010. GEMS is a 3 year case-control study assessing acute moderate-to-severe diarrhea in infants and children between 0 and 59 months of age living in western Kenya. Data on controls was not used for this analysis, and therefore methodologies related to controls will not be described.

This study presents descriptive statistics for acute diarrhea (AD), prolonged acute diarrhea (ProAD), and persistent diarrhea (PD) as each relate to age, sex, and etiologic agents. The median duration of diarrhea for age, gender, and etiologies were compared using Wilcoxon rank-sum and the Kruskal-Wallis tests. Lastly, hazard ratios were calculated for characteristics of interest using the Cox Proportional Hazards Model.

3.1 Study population

The study population included Kenyan children less than 5 years old living in the districts of Gem and Asembo in western Kenya. Annually, the target enrollment was 660 cases, usually divided among three age strata (0-11 months, 12-23 months and 24-59 months). To ensure balance amongst the age strata and account for seasonal variations based on these enrollment targets, bi-weekly enrollment was capped for each age group at 8-9 cases.

3.2 Enrollment

3.2.1 Demographic Surveillance System

A critical component to the identification of cases and controls enrolled into GEMS is the Demographic Surveillance System (DSS). Every 4 months, the DSS collects census data on each

household in a population of approximately 134,990 persons (including approximately 21,598 children under 5 years old) living in a defined study area. Information is collected on births, deaths, and in- and out- migration.

3.2.2 Case ascertainment

A child was enrolled as a case if they presented to any of 6 GEMS sentinel health centers within the DSS, lived within the DSS area, was between the ages of 0 and 59 months, and met the case definition for acute moderate-to-severe diarrhea defined as 3 or more loose stools in the previous 24 hours, within 7 days of illness onset, and with more than one of the following symptoms: sunken eyes, skin tenting, dysentery, IV rehydration, or hospitalization. All others presenting to a GEMS sentinel health centers not matching this criteria were not included in the case-control study.

3.3 Data Sources

The below section briefly describes the data collection mechanisms used during this study. Table 3.1 displays the variables used within this study.

Table 3.1. List of variables used in analysis

Variable	Coding	Type
Duration	Number 0 to 20	Continuous
Duration Type	Acute = 1, Prolonged acute = 2, Persistent = 3	Categorical
Gender	Male=1, Female=2	Categorical
Age Stratum	0-11 months, 12-23 months, 24-59 months	Categorical
Campylobacter	yes (positive identification) = 1, no=2	Categorical
Salmonella	yes (positive identification) = 1, no=2	Categorical
Shigella	yes (positive identification) = 1, no=2	Categorical
ETEC	yes (positive identification) = 1, no=2	Categorical
EPEC	yes (positive identification) = 1, no=2	Categorical
EAEC	yes (positive identification) = 1, no=2	Categorical
Rotavirus	yes (positive identification) = 1, no=2	Categorical
Giardia	yes (positive identification) = 1, no=2	Categorical
Cryptosporidium	yes (positive identification) = 1, no=2	Categorical
Bloody diarrhea	yes = 1, no = 2	Categorical
Mother's Education	LT primary school = 1, GT primary school = 2	Categorical

3.3.1 Case Report Forms (CRF)

Data for cases was collected using a series of case report forms. Information was collected on a wide range of items including eligibility criteria, non-medical characteristics of the child and their family, medical information ascertained from a clinician, laboratory results, and pre- and post-enrollment data on the health of the child reported by the caretaker.

Information important to this study, collected on the case report forms was the number of days the child experienced diarrhea prior to enrollment. At the time of the enrollment, the primary caretaker was asked: How many days including today has this episode of diarrhea lasted? Additionally, the child was ineligible for enrollment if diarrhea lasted more than 7 days. Therefore, the maximum number of days the child could have experienced diarrhea and been eligible to participate in GEMS was 7 days.

3.3.2 Memory Aid Form

In order to obtain information regarding the duration of diarrhea the caretakers were given a form to complete following enrollment, this form is referred to as the Memory Aid Form. The Memory Aid form allowed the caretaker to record whether the child has normal stool or diarrhea, up to 14 days after enrollment. At enrollment the caregiver was instructed how to complete the form correctly. The form was then reviewed and collected at the routine follow-up interview, 60 days post-enrollment.

3.3.3 Laboratory testing and stool collection

Upon enrollment, each child participating in GEMS is required to submit a stool specimen of sufficient amount. The specimen is then tested for bacterial, viral, and protozoal pathogens. Cultures were completed to test for the following bacterial pathogens: *Salmonella* spp.*, Shigella* spp.*, Campylobacter* spp.*, Aeromonas, Vibrio* spp., and *Yersinia*. Multiplex Polymerase Chain Reaction (PCR) was used to identify diarrheagenic *Escherichia coli (E. coli)*, including enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC), and enteroaggregative *E. coli* (EAggEC). Viral pathogens were identified using enzyme immunoassays and reverse transcription PCR (RT-PCR). Enzyme immunoassays were used to identify Group A rotavirus and enteric adenovirus; RT-PCR was used to identify noroviruses, sapoviruses, and astroviruses. Protozoal agents including *Entamoeba histolytica*, *Giardia lamblia, Cryptosporidium* spp. were identified using immunoassays. PCR was conducted on isolates of *Giardia* and *Cryptosporidium.*

3.4 Procedures

Memory aid forms for cases enrolled between January 31, 2008 and January 24, 2010 were sent to Atlanta, GA from the GEMS Kenya site. Binary variables were created in an Access database for each day of the memory aid form (1-14) and for the 7 days prior to enrollment. Each day, pre- and post-enrollment, was entered into an Access database. Once all diarrhea days were entered, the Access database was merged with a clean GEMS dataset and imported into JMP 8 for analysis.

For this analysis the following definitions of diarrheal duration were used: acute diarrhea (AD): 1 to 6 days of diarrhea; prolonged acute diarrhea (ProAD): 7 to 13 days of diarrhea; and, persistent diarrhea (PD): 14 or more days of diarrhea. For this study, the total duration of diarrhea was analyzed.

3.5 Analysis

Data analysis was conducted using JMP Version 8. Microsoft Excel 2007 was used for the presentation of tables and figures.

3.5.1 Descriptive statistics

Descriptive statistics were calculated for all variables. When measures of central tendency are displayed the duration of diarrhea was a continuous variable, whereas when the data is presented in groups by diarrhea type, the variable was identified as categorical.

3.5.2 Test of Medians

The Wilcoxon rank-sum test was used to calculate the difference amongst the median duration of diarrhea in males and females. And, the Kruskal-Wallis test was used to calculate the difference amongst the median duration of diarrhea based on age and etiologic agents. Both of these tests are nonparametric and were used because they do not assume the samples are normally distributed.

3.5.3 Cox Proportional Hazards Model

The Cox Proportional-Hazards Model is a form of survival analysis. Using the Cox Proportional Hazards Model has allowed us to examine the distribution of diarrhea as it is associated with covariates or risk factors. In this study the "event" occurred when diarrhea stopped. In this study, two models were used: 1) Model 1 includes age, gender, and the nine etiologic agents examined in this study and 2) Model 2 includes age, gender, etiologic agents, mother's education, and the occurrence of bloody diarrhea. For the purposes of both Model 1 and 2, age was dichotomized, with children between 0 and 11 months of age in one group and the other group consisting of children between the ages of 12 and 59 months. For Model 2, six etiologic agents were chosen based on their mean duration of diarrhea. Additionally, we chose to examine two risk factors that have been identified in the literature to cause diarrhea of longer duration, these risk factors included the mother's level of education and dysentery or bloody diarrhea. The variable for mother's education was dichotomized, one group consisting of mother's that had less than primary school education in comparison to mother's who had completed primary school or had participated in a higher level of schooling.

3.6 Ethical considerations

The following study was approved as an exempt review by Georgia State University. Additionally, GEMS has been approved under the Institutional Review Boards of the following organizations: the University of Maryland, School of Medicine, Baltimore, MD; the Centers for Disease Control and Prevention, Atlanta, GA; and, the Kenya Medical Research Institute, Kenya.

Chapter IV

RESULTS

The purpose of this study is to describe the characteristics associated with acute diarrhea (AD), prolonged acute diarrhea (ProAD), and persistent diarrhea (PD) in Kenyan children less than 5 years of age participating in the Global Enterics Multicenter Study (GEMS), by answering the following questions:

1) Is age associated with a greater likelihood of ProAD or PD compared to AD?

2) Is gender associated with a greater likelihood of ProAD or PD compared to AD?

3) Are any etiologic agents associated with a greater likelihood of ProAD or PD compared to AD?

4.1 Inclusion of cases

Between January 31, 2008 and January 24, 2010, the number of children meeting the case definition for acute moderate-to-severe diarrhea who enrolled into GEMS was 1,121. Among those children, 203 (18.1%) had missing Memory Aid forms, 32 (2.6%) had forms that were unusable due to blank or illegible entries, 3 (0.3%) had missing information on diarrhea duration prior to enrollment, and 3 (0.3%) had missing information on etiologic agent identification. Of the 880 cases with complete information, 323 (37%) children were infected with more than one etiologic agent making them not eligible for this analysis, as the focus of this study is on children with only a single etiologic agent identified on stool testing, children for whom no etiologic agent was identified on stool testing were included in the analysis as a distinct group. Therefore,

for the purposes of this analysis 557 cases were used. The above information is displayed in tabular form in Table 4.1.

Table 4.1. Inclusion of cases for analysis

Cases enrolled from January 31, 2008 - January 24, 2010 (n=1,121)	
	n (%)
Record blank or illegible	32 (2.85)
Missing memory aid forms	203 (18.11)
Missing data on diarrhea duration prior to enrollment	3 (0.27)
Missing data on pathogen identification	3 (0.27)
Total number of records not included due to missing or illegible data	241 (21.5)
Total number of cases with complete information	880
Cases with co-infection	323 (36.7)
Cases with complete information and single pathogen identified	**557**

4.2 Descriptive Statistics

4.2.1 Characteristics of study population

Among the 557 cases, 311 (55.8%) were male and 246 (44.2%) were female. Of these children 225 (40.4%) were 0-11 months of age, 148 (26.6%) were aged 12-23 months, and 184 (33.0%) were 24-59 months of age. Etiologic agents were identified in stool samples from 337 (60.5%) children, 35 (6.3%) children had a single pathogen identified in their stool, but the pathogen was identified in less than 20 cases, and therefore, was not included in this analysis. ***Giardia lamblia*** was the most frequently identified etiologic agent, found in 69 (12.4%) children, followed by enteroaggregative ***Escherichia coli (E. coli)*** identified in 48 (8.6%) children, enterotoxigeic ***E. coli*** found in 44 (7.9%) of children, rotavirus and enteropathogenic ***E. coli*** was

identified in 41 children (7.4%) each, ***Campylobacter*** spp. and ***Cryptosporidium*** spp. were both identified in 27 (4.8%) children, ***Shigella*** spp. was identified in 23 (4.1%) children, and the least frequently identified etiologic agent was ***Salmonella*** spp., found in 22 (3.9%) children.

4.2.2 Diarrhea duration

Among the 557 cases, 277 (49.7%) had AD, 242 (43.4%) had ProAD, and 38 (6.8%) had PD. On average, children experienced 7 days of diarrhea; the median duration of diarrhea was also 7 days. Table 4.2 describes characteristics of children experiencing AD, ProAD, PD, and the combined total.

Table 4.2. Characteristics of AD, ProAD, and PD displayed by age, gender, and etiology

	AD (n=277)	**ProAD (n=242)**	**PD (n=38)**	**All Cases (n=557)**
Characteristics	n (%)	n (%)	n (%)	n (%)
Gender				
Male	143 (51.6)	147 (60.7)	21 (55.3)	311 (55.8)
Female	134 (48.3)	95 (39.3)	17 (44.7)	246 (44.2)
Age				
0-11 months	93 (33.6)	111 (45.9)	21 (55.3)	225 (40.4)
12-23 months	58 (20.9)	80 (33.1)	10 (26.3)	148 (26.6)
24-59 months	126 (45.5)	51 (21.1)	7 (18.4)	184 (33.0)
Etiologic agent				
Giardia	45 (16.2)	23 (9.5)	1 (1.7)	69 (12.4)
Rotavirus	27 (9.7)	10 (4.1)	4 (6.7)	41 (7.4)
EPEC	21 (7.6)	18 (7.4)	2 (3.3)	41 (7.4)
EAEC	21 (7.6)	20 (8.3)	7 (11.7)	48 (8.6)
Campylobacter	17 (6.1)	9 (3.7)	1 (1.7)	27 (4.8)
ETEC	16 (5.8)	25 (10.3)	3 (5.0)	44 (7.9)
Salmonella	14 (5.1)	8 (3.3)	0 (0.0)	22 (3.9)
Shigella	9 (3.2)	13 (5.4)	1 (1.7)	23 (4.1)
Cryptosporidium	8 (2.9)	14 (5.8)	5 (8.3)	27 (4.8)
No pathogen identified	78 (28.2)	90 (37.2)	12 (20.0)	180 (32.3)
Single identification, <20 cases	21 (7.6)	12 (4.9)	2 (5.3)	35 (6.3)

Of cases who experienced AD, 143 (51.6%) were male and 134 (48.3%) were female, 93 (33.6%) were aged 0-11 months, 58 (20.9%) were between the ages of 12 and 23 months, and 126 (45.5%) were aged 24 to 59 months. Among those who experience ProAD, 147 (60.7%) were male and 95 (39.3%) were female, 111 (45.9%) were 0 to 11 months of age, 80 (33.1%) were aged 12 to 23 months, and 51 (21.1%) were between the ages of 24 and 59 months. Lastly, of those who experienced PD, 21 (55.3%) were male and 17 (44.7%) were female, 21 (55.3%) were aged 0 to 11 months, 10 (26.3%) were between the ages of 12 and 24 months, and 7 (18.4%) were 24 to 59 months of age. Figure 4.1 and 4.2 graphically describe the duration of diarrhea by gender and age stratum, respectively.

Figure 4.1. Duration of diarrhea by gender

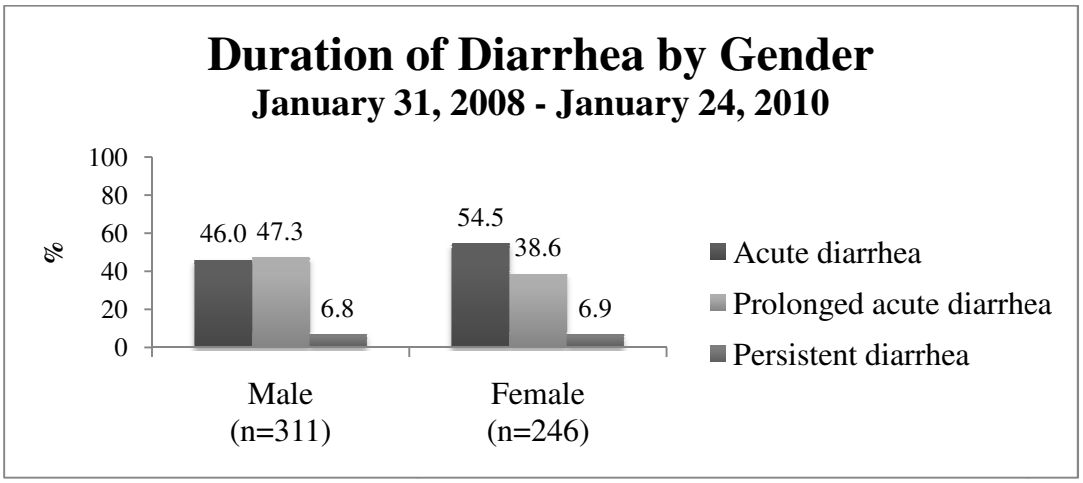
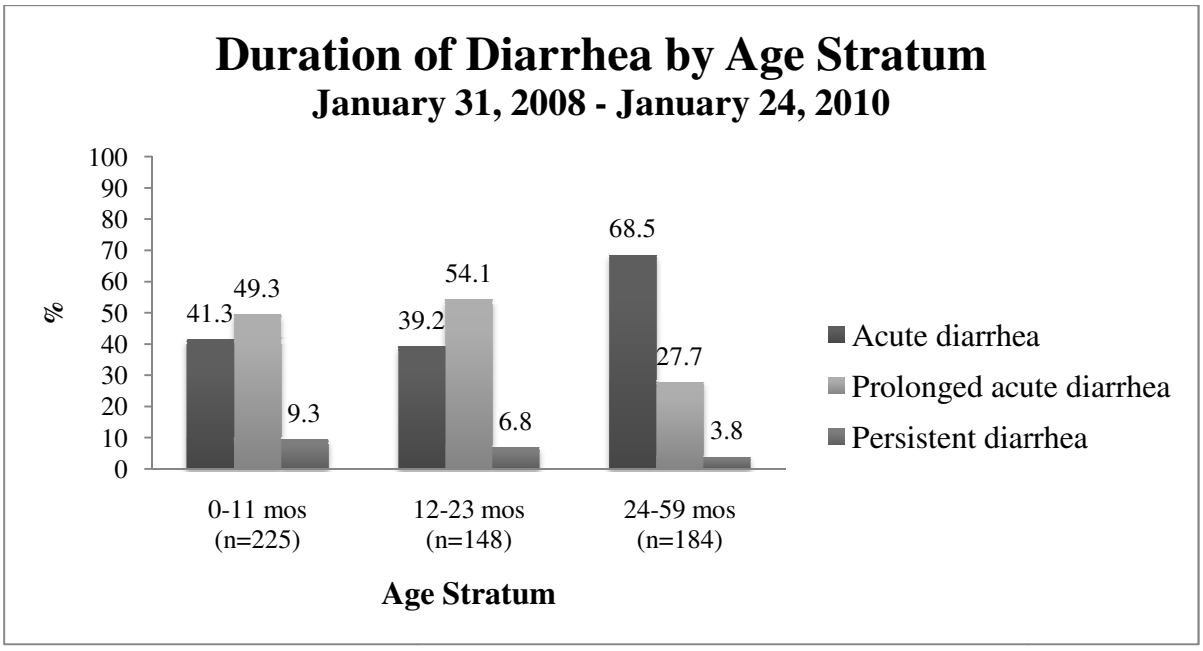


Figure 4.2. Duration of diarrhea by age stratum



A large proportion of children were found to have no pathogen identified in their stool. No pathogen was identified in the stool of 90 (37.2%) children experiencing ProAD, in 78 (28.2%) children experiencing AD, and in 12 (20.0%) children experiencing PD. The most frequently identified pathogen in children with AD was ***Giardia*** (16.2%), followed by rotavirus (9.7%), EPEC (7.6%), EAEC (7.6%), and ***Campylobacter*** (6.1%). Among children with ProAD the most frequently identified pathogens were ETEC (10.3%), ***Giardia*** (9.5%), EAEC (8.3%), EPEC (7.4%), and ***Cryptosporidium*** (5.8%). Lastly, the most frequently identified pathogens in children with PD were EAEC (11.7%), ***Cryptosporidium*** (8.3%), rotavirus (6.7%), ETEC (5.0%), and EPEC (3.3%).

4.3. Test of Medians

In order to further explore the duration of diarrhea in children as it relates to gender, age, etiology the medians of these characteristics were tested using the Wilcoxon rank-sum test and the Kruskal-Wallis Test.

Using the Wilcoxon rank-sum test the median duration of diarrhea was compared in males and females. The median duration of diarrhea for boys was 7 days and for girls was 6 days. This was not statistically significant ($P = 0.232$).

Using the Kruskal-Wallis, 1-way ANOVA, the median duration of age strata was tested. The median duration of diarrhea for infants aged 0-11 months was 7 days, for toddlers aged 12-23 months the median duration was 7 days, and for older children aged 24-59 months the median duration was 5 days. There was a significant difference amongst the age of children and the duration of diarrhea ($P = <.0001$). This test reveals there is a difference somewhere in the three age strata, but does not identify where the differences are.

To compare the duration of diarrhea amongst etiologic agents the Kruskal-Wallis, 1-way ANOVA test was performed. The ANOVA test revealed a significant association between the median duration of diarrhea and the pathogens identified ($P = 0.0012$), but again this does not identify where the association lies. Etiologic agents ranked from longest to shortest median duration of diarrhea are ***Cryptosporidium*** (9 days), ***Shigella*** (9 days), ETEC (7.5 days), EAEC (7 days), EPEC (6 days), ***Salmonella*** (6 days), Rotavirus (6 days), ***Campylobacter*** (6 days), and ***Giardia*** (6 days).

4.4. Cox's Proportional-Hazards Model

Model 1 shown in Table 4.3 displays the characteristics included in the model and their associated risk ratios and confidence intervals calculated using Cox's Proportional-Hazards Model. These results indicate children between the ages of 0 and 11 months have a 1.3 times greater likelihood of experiencing diarrhea of longer duration. Additionally, children infected with ***Cryptosporidium*** have a 1.5 times greater likelihood of experiencing diarrhea of extended duration. All other factors in this model were not significant.

Table 4.3. Cox Proportional Hazards, Model 1

Model	Variable	Risk Ratio	Confidence Interval
Model 1	Age (0-11 mos, 12-59 mos)	1.34	1.119, 1.603
	Gender (Male=1, Female=2)	1.09	0.918, 1.292
	Campylobacter (1=Yes, 2=No)	0.76	0.518, 1.166
	Salmonella (1=yes, 2=no)	0.83	0.546, 2.270
	Shigella (1=yes, 2=no)	1.26	0.835, 1.989
	ETEC (1=yes, 2=no)	1.13	0.825, 1.581
	EPEC (1=yes, 2=no)	0.94	0.674, 1.331
	EAEC (1=yes, 2=no)	1.21	0.889, 1.692
	Rotavirus (1=yes, 2=no)	0.87	0.624, 1.236
	Giardia (1=yes, 2=no)	0.79	0.602, 1.047
	Cryptosporidium (1=yes, 2=no)	1.53	1.043, 2.344

Model 2 (Table 4.4) includes a number of different covariates than Model 1. However, the results are very similar. In Model 2, only two covariates were statistically significant, which again were age and infection with ***Cryptosporidium***. The risk ratio for age was the same, children between the ages of 0 and 11 months had a 1.3 times greater likelihood of experiencing diarrhea of longer duration. For children infected with ***Cryptosporidium*** their risk increased slightly, to 1.7 times more likely than those not infected with ***Cryptosporidium*** to experience diarrhea of greater length.

Table 4.4. Cox Proportional Hazards, Model 2

Model	Variable	Risk Ratio	Confidence Interval
Model 2	Age (0-11 mos, 12-59 mos)	1.34	1.125, 1.599
	Gender (1=Male, 2=Female)	1.1	0.930, 1.307
	Bloody diarrhea (1=yes, 2=no)	1.04	0.791, 1.399
	Mother's Education (1=LT primary school, 2=GT primary school)	0.99	0.840, 1.181
	Salmonella (1=yes, 2=no)	0.89	0.598, 1.431
	Shigella (1=yes, 2=no)	1.35	0.903, 2.129
	ETEC (1=yes, 2=no)	1.22	0.815, 1.689
	EPEC (1=yes, 2=no)	1.01	0.736, 1.425
	EAEC (1=yes, 2=no)	1.31	0.732, 1.811
	Cryptosporidium (1=yes, 2=no)	1.66	1.141, 1.097

4.5. Summary of results

To summarize, we found no association amongst gender and the length of diarrhea. However, we found that age was associated with diarrhea of extended duration. Children aged 0 to 11 months were 1.3 times more likely to experience diarrhea of longer duration than their counterparts. Lastly, based on this analysis, which included nine pathogens identified in more than 20 children, we found only ***Cryptosporidium*** to be more associated with ProAD and PD. Children infected with ***Cryptosporidium*** were 1.5 to 1.7 times more likely to have diarrhea with of longer duration than their counterparts.

Chapter V

DISCUSSION AND CONCLUSION

The purpose of this study was to describe the characteristics associated with acute diarrhea (AD), prolonged acute diarrhea (ProAD), and persistent diarrhea (PD) in Kenyan children less than 5 years of age participating in the Global Enterics Multicenter Study (GEMS). These characteristics have been described and the associations identified based on the statistical analysis used in this study. The major findings, limitations, recommendations, and conclusions are summarized below.

5.1 Gender

In this study we set out to identify if gender was associated with a greater likelihood of ProAD or PD compared to AD. We found that there was no association between AD, ProAD, or PD. This is consistent with other studies exploring the relationship of gender and diarrhea of extended duration.[16,22,23]

5.2 Age

We also wanted to identify if age was associated with a greater likelihood of ProAD or PD compared to AD. We found that age was associated with diarrhea of extended duration. Based on previous literature identifying younger children as the most at risk group,[11,15,16,22-25] age was dichotomized using infants (0-11 months) as one group and children aged 12 to 59 months as the comparison group in the Cox Proportional-Hazards Model. In both models age was found to have a statistically significant association with the duration of diarrhea and children less than or equal to 11 months of age were 1.3 times more likely to experience diarrhea of longer duration than their counterparts.

This is consistent with previous literature. [11,15,16,22-25] Most studies have identified the first 6 months of life to be the most significantly associated with diarrhea of extended duration.

This suggests younger children are potentially at greater risk for longer, more severe illness possibly because their immune systems are less able to control gastrointestinal infections. When tailoring interventions and programs, the focus should be more heavily concentrated on these children.

5.3 Etiologic agents

Another objective of this study was to identify etiologic agents that were more associated with ProAD or PD compared to AD. In this analysis, we included nine pathogens that were found in more than 20 children. Of these pathogens, we found only ***Cryptosporidium*** to be more associated with ProAD and PD. Based on our models, ***Cryptosporidium*** was found to be 1.5 to 1.7 times more likely to cause diarrhea with a longer duration. Similar to these results, previous literature has identified ***Cryptosporidium*** as an important pathogen in the epidemiology of PD.[7,12]

5.4 Study Limitations

This study has a number of limitations. First, we did not include children who had more than one pathogen isolated from their stool. A large proportion of children, 36% had more than one pathogen identified in their stool. Clearly, much of this population had multiple infections and this needs to be studied further, but was beyond the scope of this paper. Second, we analyzed the total number of days the child experienced diarrhea, rather than examining a defined episode. We also assumed the number of days of diarrhea the caretaker reported prior to enrollment were consecutive. Third, we did not ascertain information related to previous illness which has been found to be a predictor of PD. [7,10] Lastly, this study is limited in its generalizablity to other populations.

5.5 Recommendations

Based on our findings, interventions related to diarrhea and diarrhea of extended duration should focus more closely on young children, especially children less than 24 months of age. This study found that *Cryprospordium* was more associated with diarrhea of longer duration than other etiologic agents, however previous research suggests a wide variation among pathogens and the duration of diarrhea. It is valuable to know that in this population *Cryptosporidium* has been associated with diarrhea of longer duration, but this association should be studied further. Additionally, further analyses should be conducted to explore differences amongst age and gender by the various enteric pathogens.

In general, when children have diarrhea it should be recommended that parents understand the importance of rehydration, and the use of oral rehydration salts (ORS) should be encouraged. The WHO guidelines for preventing diarrhea should also be promoted to the community and to parents, these recommendations include: improved access to water and sanitation, increased handwashing with soap, promotion of exclusive breastfeeding for the first 6 months of life, improved nutrition to decrease the number of children who are malnourished, increased use of Vitamin A and zinc, and finally, increased rates of immunization against both rotavirus and measles.[1]

More research should focus on diarrhea of extended duration. In particular, research should focus on potential risk factors of ProAD and PD which may include malnutrition, time of weaning, bloody stools, maternal education, and the time at which a child experiences the first episode of diarrhea. Research should also focus on the consequences of ProAD and PD, such as nutritional deficiencies, delays in growth, and the impact on cognitive function. The length of diarrhea should also be examined in children with multiple pathogens identified in their stools.

Studies of this nature should be expanded across geographic locations and this data should be compared across all GEMS sites using similar methodologies.

5.6 Conclusion

In conclusion, this study has found that children less than 1 year old and children infected with *Cryptosporidium* are at a greater risk for diarrhea of extended duration. However, there was no association between gender and the length of diarrhea. Diarrhea of extended duration has been found to lead to adverse health outcomes, including: delays in growth,[6] nutritional deficiencies,[7] and decreased cognitive function over time.[7,8] In order to better understand diarrhea of extended duration and how to prevent these adverse outcomes, future research should focus on the characteristics influencing ProAD and PD. This information will be valuable in preventing morbidity and mortality associated with diarrheal diseases.

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