



The epidemiology of diarrhea: Determination of the burden, etiology and consequences of diarrheal disease in children aged 0-59 months in Manhiça District, Mozambique

La epidemiología de las diarreas: Determinación del peso, etiología y secuelas de la enfermedad diarreica en niños de 0-59 meses de edad en el Distrito de Manhiça, Mozambique

Tacilta Helena Francisco Nhampossa

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TESIS DOCTORAL

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La epidemiología de las diarreas: Determinación del peso, etiología y secuelas de la enfermedad diarreaica en niños de 0-59 meses de edad en el Distrito de Manhiça, Mozambique

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para optar al grado de Doctor en Medicina

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25 de Octubre, 2013

*To
God
My parents and family
CISM*

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1. GLOSSARY

AF	Attributable fraction
BCG	Bacillus Calmette Guérin
CFR	Case fatality rates
CISM	Centro de Investigação em Saúde da Manhiça
CH	Central Hospital
CHWs	Networks of community health workers
CRESIB	Centre de Recerca en Salut Internacional de Barcelona
CTX	Cholera toxin
DAEC	Diffusely adherent <i>E. coli</i>
DCC	Data Coordinating Center
DSS	Demographic surveillance system
EAEC	Enterogastric <i>E. coli</i>
EHEC	Enterohemorrhagic or verotoxigenic <i>E. coli</i>
EPEC	Enteropathogenic <i>E. coli</i>
ETEC	Enterotoxigenic <i>E. coli</i>
HIV/AIDS	Human immunodeficiency virus/acquired immune deficiency syndrome
HU	Health Unit
LMIC	Low and middle-income countries
LT	Heat-labile enterotoxin
MDH	Manhiça District Hospital
MISAU	Ministério da Saúde
MSD	Moderate-to-severe diarrhea
MUAC	Mid-upper arm circumference
NHS	National Health Service

ORS	Oral rehydration solution
PCR	Polymerase chain reaction
RT-PCR	Real time-polymerase chain reaction
SAM	Severe acute malnutrition
SD	Standard deviation
ST	Heat-stable enterotoxin
UNICEF	United Nations Children's Fund
USA	United States of America
WHO	World Health Organization
ZOT	Zonula occludes toxin

2. SUMMARY (CASTELLANO)

Las enfermedades diarreicas siguen siendo una de las principales causas de morbi-mortalidad entre los niños de menos de cinco años en los países en desarrollo. A nivel mundial, los niños menores de cinco años experimentan, en promedio, 3.2 episodios de diarrea cada año. Esta enorme carga se traduce en 800,000 muertes infantiles anuales por diarrea en este grupo de edad, lo que representa hasta un 11% de la carga total de las muertes pediátricas. El impacto de las enfermedades diarreicas es particularmente flagrante en el África subsahariana y en el sudeste de Asia, dónde se concentran hasta un 80% de todas las muertes.

Del mismo modo, la malnutrición es muy frecuente en los países en vías de desarrollo y se considera que es causa subyacente de hasta un tercio de las muertes anuales que se producen en niños menores de cinco años. Ambas enfermedades están extraordinariamente ligadas, y actualmente se considera que la malnutrición es un importante factor de riesgo (y por tanto contribuye de forma importante a la carga global) de las enfermedades diarreicas, principalmente como consecuencia del impacto negativo en la función inmune del huésped que reduce la resistencia a organismos infecciosos.

En este sentido, y asumiendo la elevada carga global de tanto las enfermedades diarreicas como de la malnutrición en los países en vías de desarrollo, parece urgente mejorar el manejo y la prevención de estas enfermedades; y sobre todo fomentar la implementación de aquellas intervenciones con probada eficacia para reducir su inaceptable impacto. Para este fin, y con el objetivo de orientar la implementación de estrategias de tratamiento y establecer áreas prioritarias de intervenciones, los responsables de políticas de salud pública requieren información específica precisa sobre la carga, la etiología y las secuelas de

las enfermedades diarreicas (incluida la malnutrición) en cada uno de los diferentes países y ambientes epidemiológicos donde estas enfermedades son altamente incidentes. Esta tesis pretende, a través de los diferentes estudios aquí englobados, dar respuesta a esa necesidad providenciando una serie de datos, complementarios en su conjunto, que permitirán obtener una visión global y adecuada de la situación actual de las enfermedades diarreicas y la malnutrición en una zona rural de Mozambique, uno de los países más pobres del África sub-sahariana.

Esta tesis está basada en el trabajo realizado a través de una colaboración entre el *Centro de Investigação em Saúde da Manhica* en Mozambique, el *Centre de Recerca en Salut Internacional de Barcelona* (CRESIB), en España; y el *Center for Vaccine development* de la facultad de medicina de la Universidad de Maryland, en Estados Unidos. Los cinco artículos de esta tesis surgen de diferentes (pero complementarios) proyectos de investigación, todos ellos relacionados con las enfermedades diarreicas pediátricas en países en desarrollo. Estos proyectos abarcan desde la investigación social básica de los determinantes del uso de servicios de salud y control de enfermedades en caso de enfermedad diarreica, hasta análisis más específicos de los factores de riesgo y los determinantes microbiológicos de la enfermedad.

La primera sección de esta tesis describe los resultados de un ambicioso estudio multicéntrico (Estudio “GEMS”), diseñado como un estudio de casos y controles para averiguar la carga de enfermedad, factores de riesgo microbiológico, etiología y presentación clínica de los episodios de diarrea (moderada a grave en términos de gravedad) detectados en niños de 0-59 meses de edad entre diciembre de 2007 y octubre de 2011 en cuatro países de África subsahariana (Kenya, Malí, Mozambique, Gambia) y tres más en el

sudeste de Asia (Bangladesh, India, Pakistán). Los tres primeros artículos de esta tesis describen los resultados generales (primer artículo), y los resultados específicos de Mozambique (artículos 2 y 3).

El primer artículo resume los resultados del análisis combinado de los datos recogidos en el estudio multicéntrico realizado en los siete países mencionados anteriormente durante los primeros tres años del estudio. Durante este período, la incidencia estimada de diarrea moderada a grave fue mayor en la India, seguida de Kenia y Malí, siendo la más baja detectada en Gambia, Pakistán, Bangladesh y Mozambique. La incidencia anual global de diarrea moderada a grave por 100 niños-año fue de 30.8 nuevos episodios (IC 95%: 24.8-36.8) en los niños con edad 0-11 meses; 23.1 (IC 95%: 17.2-29.0) para los niños con edad 12-23 meses y finalmente de 7.7 (IC del 95% 3.9-11.5) para los niños mayores (24-59 meses). Mediante el análisis ajustado de fracciones atribuibles poblacionales, pudo estimarse que la mayoría de los casos de diarrea moderada a grave fueron debidos a cuatro patógenos: rotavirus, *Cryptosporidium*, ETEC ST (ST sólo o ST / LT) y *Shigella*. Otros patógenos fueron específicamente incidentes en algunos de los países del estudio, pero no en todos, como por ejemplo *Aeromonas*, *Vibrio cholerae* O1 o *Campylobacter jejuni*. Las probabilidades de morir durante el seguimiento fueron 8.5 veces superiores en aquellos pacientes con diarrea moderada a grave que en sus respectivos controles (odds ratio 8.5 (IC del 95%: 5.8-12.5, $p < 0,0001$). La mayoría de las muertes (167/190 (87,9 %)) se produjo en aquellos pacientes menores de 2 años de vida. Los patógenos asociados con mayor riesgo de muerte fueron el *Escherichia coli* enterotoxigénica expresando toxina termo-estable (hazard ratio [HR] 1.9; 95%IC 0.99-3.5) y el *Escherichia coli* típicamente enteropatogénico (HR 2.6 ; 1.6-4.1) en niños de 0-11 meses; así como el *Cryptosporidium* (HR 2.3 ; 1.3-4.3) en los niños con edades entre los 12 y 23 meses .

El segundo artículo describe las tendencias históricas de la incidencia y carga de las enfermedades diarreicas y caracteriza la etiología microbiológica de la diarrea moderada a grave entre los niños que viven en el distrito de Manhica (Mozambique). Este estudio demuestra que la incidencia de la diarrea aguda ha disminuido en alrededor del 80% durante el período de 2001 a 2012. La incidencia de diarrea moderada a grave por cada 100 años-niño a riesgo durante el global del período 2007-2011 fue de 9.85, 7.73 y 2.10 para los niños de 0-11, 12-23 y de 24-59 meses, respectivamente. Mediante el análisis ajustado de fracciones atribuibles poblacionales, la mayoría de los casos de diarrea de moderada a grave fueron de nuevo debido a rotavirus, *Cryptosporidium*, ETEC ST (ST sólo o ST / LT), *Shigella* y Adenovirus 40/41.

Los resultados de los factores de riesgo asociados con la aparición de diarrea moderada a grave entre los niños que viven en el distrito de Manhica (Mozambique) se presentan en el tercer artículo y muestran que tener un cuidador diferente de la madre y beber agua almacenada fueran factores de riesgo de episodios de diarrea moderada a grave. Por otro lado, lavarse las manos regularmente sobre todo después de manipular animales o antes de preparar la comida del bebé, y tener facilidades para disponer las heces del niño son factores de protección para la diarrea moderada a grave. Sin embargo, el riesgo de diarrea moderada a grave no se ha mostrado en este estudio asociado con los indicadores económicos de los hogares, ni tampoco con el nivel educativo del cuidador.

La segunda parte de esta tesis se basa en dos encuestas realizadas en la comunidad de Manhica acerca de las actitudes y la utilización de servicios de salud en caso de diarrea. Estas encuestas fueron realizadas durante el estudio de casos y controles que se ha descrito anteriormente, a través de entrevistas realizadas a los principales cuidadores de niños de 0 a

59 meses residentes en el distrito de Manhiça. El cuarto artículo de esta tesis presente los resultados de estas encuestas. Una importante proporción de los cuidadores que reportaron un episodio de diarrea durante las dos semanas previas a la entrevista (65.2% en la primera encuesta y 43.8 % en el segunda encuesta) informaron que acudieron a un centro de salud. Asimismo, el uso de los servicios de salud en caso de diarrea pudo asociarse a una necesidad percibida y a un bajo conocimiento de los signos de deshidratación; pudiendo haber sido obstaculizado, paradójicamente, por la situación económica. El conocimiento de la comunidad acerca de la enfermedad, sus manifestaciones clínicas, y los factores de riesgo asociados con la gravedad fue adecuado, contrariamente al conocimiento de aquellas prácticas más adecuadas para el tratamiento de estos episodios, como por ejemplo, la recomendación de aumentar la ingesta de líquidos.

El último artículo de esta tesis describe los resultados de un análisis retrospectivo de datos recogidos a través del sistema de vigilancia de morbilidad de los casos de malnutrición detectados en niños menores de cinco años de edad atendidos en el Hospital Distrital de Manhiça durante el período de 2001 a 2010. Durante los 10 años de vigilancia, de los 274,813 niños atendidos en las consultas externas del Hospital Distrital de Manhiça, casi la mitad (47.0%) presentó indicios de malnutrición, una parte importante de los cuáles (6%; 17,188/274,813) presentando criterios de malnutrición grave. De éstos, sólo el 15% (2,522 /17,188) fueron finalmente admitidos. La tasa de letalidad asociada a la malnutrición grave fue del 7% (162/2,274). Algunos factores, como la bacteriemia, hipoglucemia, candidiasis oral, edema, palidez, respiración profunda y diarrea aguda, se asociaran de forma independiente con un mayor riesgo de mortalidad en el hospital, mientras que la malaria y el aumento de la edad se asociaron de forma independiente con un menor riesgo de mal pronóstico. En general las tasas de incidencia mínima comunitarias fueron 15 casos por cada

1000 niños-año a riesgo, y los niños de 12-23 meses de edad presentaron la incidencia más alta.

Esta tesis presenta, por tanto, una visión integral de la etiología, factores de riesgo y las características clínicas de la enfermedad diarreica y la malnutrición, junto con un análisis de sus factores de riesgo y determinantes socioeconómicos asociados. Los resultados aquí presentados son de gran utilidad desde el punto de vista de salud pública, y deberían servir a los responsables políticos para tomar medidas basadas en la evidencia y disminuir así la inaceptable morbi-mortalidad todavía asociada con este tipo de enfermedades.

3. SUMMARY (ENGLISH)

Diarrheal diseases remain a major contributor to illness and death among children less than five years in developing countries. Globally, children aged less than five years experience on average of 3.2 episodes of diarrhea every year. Such an enormous burden is translated into 800,000 annual child deaths from diarrhea in this age group, representing up to 11% of the total burden of pediatric deaths. The impact of diarrheal diseases is particularly blatant in Sub-Saharan Africa and Southeast Asia accounting for more than 80% of all deaths.

Similarly, malnutrition is highly prevalent in developing countries and is considered to be the cause of up to a third of the annual deaths occurring in children under the age of five. Potentially, malnutrition is believed to play a key role to the high burden of diarrheal diseases due to the negative impact in host immune function which reduces resistance to infectious organisms.

Acknowledging the high burden of diarrheal disease and malnutrition in developing countries, boosting the implementation of existing control measures interventions to prevent disease and improve outcomes is desirable. However, in order to guide deployment of effective prevention and treatment strategies and target appropriate interventions, public health policy makers require accurate information on the burden, etiology and *sequelae* of diarrheal disease (including malnutrition) from such developing countries and epidemiologic settings. The scope of the work that is the basis for this thesis is to respond to such necessity with complementary data that will allow obtaining an overall adequate picture of the current situation of diarrheal disease and malnutrition in a Mozambican rural area, one of the poorest sub-Saharan African countries.

This thesis is based on work undertaken through a partnership between the *Centro de Investigação em Saúde da Manhica* in Mozambique, the Barcelona Center for International Health Research, in Spain and the Center for Vaccine Development at the University Of Maryland School Of Medicine, in USA. The five articles of this thesis were produced within different but complementary areas of research all related with diarrheal disease in children less than five years of life living in developing countries and ranges from the basic social investigation of the determinants of use of health services and disease management in case of diarrheal illness, to the most specific analysis of risk and microbiological determinants of the disease.

The first area of research includes the development of a multicenter case-control study /the “GEMS” study) describing the burden of disease, risk factors, microbiologic etiology and clinical presentation of moderate-to-severe diarrhea among children aged 0-59 months between December 2007 and October 2011 in seven countries of sub-Sahara Africa (Kenya, Mali, Mozambique, The Gambia) and South-East Asia (Bangladesh, India, Pakistan). The first three articles from this thesis describe the general results (first paper), and site-specific (Mozambique) results (papers 2 and 3).

The first paper presents results of a multicenter analysis of data collected from the seven sites during the first three years of the study. During this period, the estimated incidence of moderate-to-severe diarrhea was highest in India, next highest in Kenya and Mali, and lowest in The Gambia, Pakistan, Bangladesh, and Mozambique. The overall annual incidence of moderate-to-severe diarrhea per 100 child-years was 30.8 (95% CI 24.8–36.8) for infants, 23.1 (95% CI 17.2–29.0) for toddlers, and 7.7 (95% CI 3.9–11.5) for children. By analyzing adjusted population attributable fractions, most attributable cases of moderate-

to-severe diarrhea were due to four pathogens: rotavirus, *Cryptosporidium*, ETEC ST (ST only or ST/LT) and *Shigella*. Other pathogens were important in selected sites (eg, *Aeromonas*, *Vibrio cholerae* O1, *Campylobacter jejuni*). Odds of dying during follow-up were 8.5-fold higher in patients with moderate-to-severe diarrhea than in controls (odds ratio 8.5, 95% CI 5.8–12.5, $p < 0.0001$); most deaths (167 [87.9%]) occurred during the first 2 years of life. Pathogens associated with increased risk of case death were ST-ETEC (hazard ratio [HR] 1.9; 0.99–3.5) and typical enteropathogenic *E coli* (HR 2.6; 1.6–4.1) in infants aged 0–11 months, and *Cryptosporidium* (HR 2.3; 1.3–4.3) in toddlers aged 12–23 months.

The second paper describes historical incidence trends and the burden of diarrheal diseases and characterizes microbiologic etiology of moderate-to-severe diarrhea among the children living in Manhiça district (Mozambique site). Herein, the results demonstrate that the incidence of acute diarrhea has dropped by about 80% over the period 2001-2012. Incidence of moderate-to-severe diarrhea per 100 child years at risk for the period 2007-2011 was 9.85, 7.73 and 2.10 for children aged 0-11, 12-23 and 24-59 months respectively. By analyzing adjusted population attributable fractions, most cases of moderate-to-severe diarrhea were again due to rotavirus, *Cryptosporidium*, ETEC ST (ST only or ST/LT), *Shigella* and Adenovirus 40/41.

The results of the risk factors associated with the occurrence of moderate-to-severe diarrhea among the children living in Manhiça district (Mozambique) are presented in the third paper and illustrate that having a caretaker who was not the mother and giving stored water were independent risk factors for moderate-to-severe diarrhea. On the other hand, regular washing hands particularly after handling animals or before preparing baby's food, and having facilities to dispose child's stool were protective factors for moderate-to-severe diarrhea.

Risk of moderate-to-severe diarrhea was not found to be strongly associated with economic indicators of the households and education level of the caretaker.

The second section of this thesis is based on two community surveys about attitudes and health care utilization in case of diarrhea performed during the above described case-control study, through interviews conducted with primary caretakers of children aged 0-59 months living in Manhiça district. The fourth paper of this thesis is a result of these surveys of health-care in case of diarrhea. Of those primary caretakers reporting an episode of diarrhea during the recall period, 65.2% in first survey and 43.8% in second survey reported seeking care at a health facility. The use of health facilities in case of diarrhea was found to be fundamentally associated with the perceived need, lower knowledge of dehydration signs and may have been hampered by the economic status. Community knowledge of the disease, its manifestations and the risk factors associated to severity seemed adequate, contrarily to those regarding best practices to treat such episodes, such as for instance the recommendation of increasing liquid intake.

The last paper of this thesis describes a retrospective analysis of data recorded through the health facility morbidity surveillance system of all malnutrition cases in children aged less than five years of age seen at Manhiça's District Hospital during the period 2001 to 2010. During the 10 year-long study surveillance, 274,813 children were seen at the outpatient clinic of Manhiça's District Hospital, almost half of which (47.0%) presenting with some indication of malnutrition, and 6% (17,188/274,813) with severe malnutrition. Of these, only 15% (2,522/17,188) were eventually admitted. Case fatality rate of severe malnutrition was 7% (162/2274). Bacteremia, hypoglycemia, oral candidiasis, edema, pallor, deep breathing and acute diarrhea were independently associated with an increased risk of in-hospital

mortality, while malaria parasitaemia and increasing age were independently associated with a lower risk of a poor outcome. Overall Minimum Community-based Incidence rates were 15 cases per 1000 child-years, and children aged 12-23 months of age had the highest incidence.

Thus, this thesis presents a comprehensive vision of the etiology, risk factors and clinical characteristics of pediatric diarrheal disease and malnutrition, together with an analysis of its associated risk factors and socio-economic determinants, the results of which may be of great public health utility for policy makers in order to decrease the unacceptable morbidity and mortality still associated with such diseases.

4. GENERAL INTRODUCTION

4.1. Diarrheal disease

4.1.1 Case-definition, and Global burden and distribution of diarrheal disease

Diarrhea can be described as the passing of unusually loose or liquid stools (1). It is generally defined as three or more loose or watery stools within a 24-hour period, or a decrease in the consistency of the stool from that which is normal for the patient (2, 3).

In the absence of demonstrable causal forces, many descriptive terms have arisen through the years. Names such as “Montezuma’s revenge,” “Delhi belly,” “Aden gut,” “gyppi tummy,” “Aztec two-step,” “Greek gallop,” “Rome runs,” “Hong Kong dog,” “Turkey trots,” “La turista,” “Basra belly,” and “back door sprint” (similarly “mavabswi ya ku buyelela,” which means “go and come back” in Xangana, a language spoken in Southern Mozambique) illustrate its wide spread occurrence (4). Nevertheless only in 1958, the World Health Organization recognized diarrheal disease as “a major health problem” (5). In addition, reducing mortality in children was explicitly acknowledged as a priority insofar as “practical programs cannot be completed without consideration of methods for prevention of death in children (5).” Since then, investigations to address diarrheal disease risk factors have been conducted in different regions of the world. With a better knowledge of the determinants of disease, strategies to reduce the burden of diarrheal disease were launched worldwide. As a result, global estimations of the number of diarrhea related deaths in children under five have shown a steady decline, from 4.6 million in the 1980s (6), to 3.3 million in the 1990s (7), 2.5 million in the year 2000 (8), 1.35 million in 2008 (9, 10) and finally 0.8 million in 2010 (11).

However, despite the above reports of an important death decline, diarrheal disease continues to be a health problem and remains the second most common infectious cause of mortality among children under five years of age. Globally, about 11% of the total burden of

pediatric deaths in children younger than 5 of age is currently attributed to diarrheal disease (figure 1) (11). Lozano et al demonstrated that diarrheal disease accounts for 2.7% in children aged 0-27 days, 17.4 % in children aged 18-365 days and 11.9% in children aged 1-4 years of the total deaths in the respective age group and the majority of deaths were attributed to diarrheal disease caused by rotavirus and *Cryptosporidium* in all age groups (12).

Even greater than the mortality is the serious morbidity from diarrheal diseases that has not shown a parallel decline. Every year, children aged less than five years experience an average of 3.2 episodes of diarrhea corresponding to about 2-4 billion cases of diarrheal disease *per year* (8, 13-16).

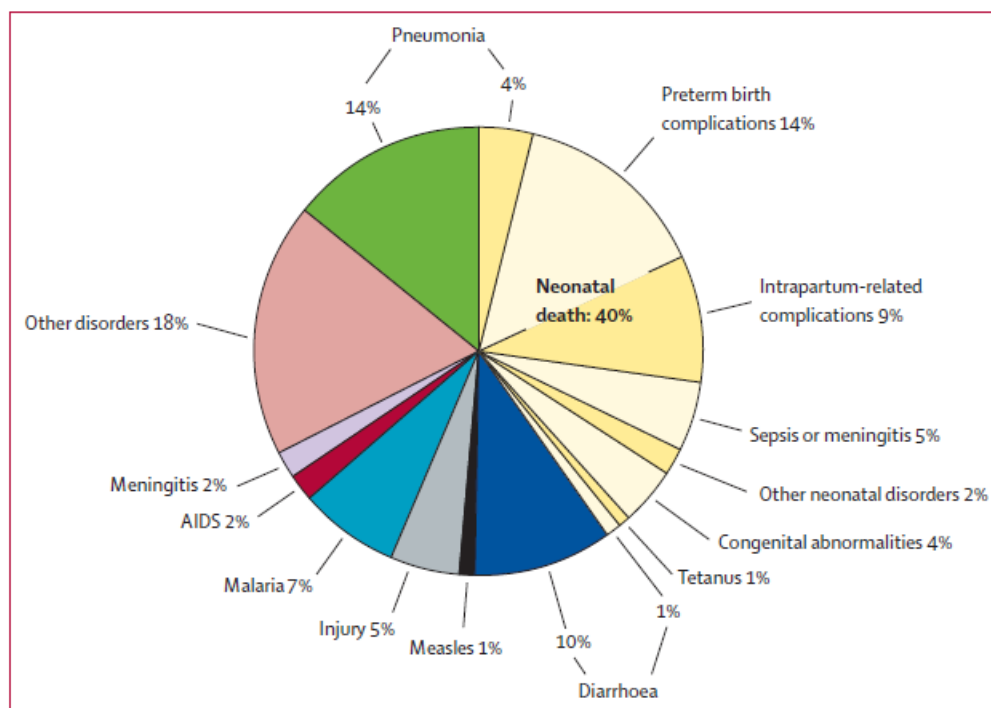


Figure 1: Principal causes of death among children <5 years of age globally, (figure from Liu et al, Lancet 2012)

The geographic distribution of diarrheal disease and its associated deaths however is very unbalanced and the poorest countries are the most affected. About 80-90% of all diarrhea related-deaths in children younger than 5 years occur in Sub-Saharan Africa and South-East Asia (17-19). Estimates are that diarrheal disease accounts for 11% in Africa and Southeast Asia and 12% in Eastern Mediterranean of the total deaths by region (11). Quite the opposite, mortality in the more developed regions has been reduced to very low levels; only 4% in Europe and in the regions of North America (11).

It is pertinent to mention that worldwide diarrheal disease burden estimation relies primarily on mortality and morbidity data; however despite several attempts to estimate mortality from diarrheal disease over the past decades and in recent years, the uncertainty surrounding its current level remains quite high. This occurs partly because of the poor and scarcely available data, but also on account of the lack of consistency in methods utilized to study such a disease. Data are very scarce in low-income settings where they are most needed and estimations are necessary for these areas.

4.1.2 Risk factors for diarrheal disease

Over the last decades, several reports have identified socioeconomic, host susceptibility and seasonality characteristics as risk factors for diarrheal morbidity and mortality (17, 20).

Socioeconomic factors

Poverty and mother's education have been considered basic elements, among socioeconomic factors because they are indicators of resource availability and knowledge or behavior in relation to child health. According to the literature, mothers with higher educational levels have more knowledge and give greater importance to breast-feeding, to appropriate feeding practices, to use of health services, to cleanliness and hygienic habits of the house and

relating to the child such as hygienic disposal of feces, washing hands after defecation, after handling feces or before handling food and storing food/water conditions that reduce the level of exposure to pathogens and all of which increase the child's resistance against infectious diseases (21-27).

Host susceptibility factors

Diarrhea and related-deaths have their peak incidence in the period from the first month of life until the second year of life, overlapping with the transition from exclusive breastfeeding to the introduction of external food, and interacting with exposure to contaminated food and to lack of sanitation and personal and domestic hygiene. Additionally, between this period children are biologically susceptible because their immune systems are still developing and would have developed few antibodies to fight infections (28). Diarrheal morbidity and mortality rates tend to decline progressively after 24 months of age (29). Malnutrition and infections such as diarrhea are bi-directionally related. Infection adversely affects nutritional status through reductions in dietary intake and intestinal absorption, increased catabolism and sequestration of nutrients that are required for tissue synthesis and growth. On the other hand, malnutrition can predispose to infection because of its negative impact on the barrier protection afforded by the skin and mucous membranes and by inducing alterations in host immune function and infection suppression (30, 31). As infections can predispose to diarrhea, presumably as a result from immunological impairment; consequently, failure to get immunized for those infections such as measles increase the risk of diarrhea (32). Nowadays, the emergence and spread of the HIV/AIDS pandemic in the poorest countries has been implicated as a huge contributor to diarrheal morbidity and mortality (33-36). In recent years, the basis for the protection conferred by natural bacterial flora in the intestinal tract has also been investigated by many

studies (37), but to date no clear assessment of the role of the gut microbiota on preventing/accelerating diarrheal disease has been proposed.

Seasonality factors

Distinct seasonal patterns of diarrhea occur in many geographical areas. In temperate climates, bacterial diarrheas occur more frequently during the warm season, whereas viral diarrheas, particularly diseases caused by rotavirus, peak during the winter. In tropical areas, rotavirus diarrhea occurs throughout the year, increasing its frequency during drier, cool months, whereas bacterial diarrheas increase during the warm season with rainfall. The incidence of persistent diarrhea follows the same seasonal pattern of that of acute watery diarrhea (20, 38).

4.1.3 The etiology and transmission of diarrheal disease

Diarrhea may be caused by infectious organisms, including viruses, bacteria, and parasites that are transmitted from the stool of one individual to the mouth of another, termed *fecal-oral transmission*. Some are well known, others are recently discovered or emerging new agents, and presumably many remain to be identified. They differ in the route from the stool to the mouth and in the number of organisms needed to cause infection and illness. Certain enteropathogens are adapted to infect animals and pose no threat to humans, and others are adapted to humans and do not infect animals. The majority, however, are not adapted to a specific host and can infect either humans or domestic animals, thus facilitating transmission of these organisms to humans (17). The most common pathogens include, among others, Rotavirus, *Salmonella spp.*, *Escherichia coli*, *Shigella spp.*, *Campylobacter jejuni*, *Giardia lamblia*, *Cryptosporidium parvum* and *Entamoeba histolytica* (39, 40).

Viruses

The most frequent cause of viral diarrhea is, indisputably, the rotavirus. Other frequent causes of viral diarrhea include enteric adenovirus, calicivirus, astrovirus, norovirus (Norwalk-like viruses) and enterovirus. In general they can be detected in outbreaks (calicivirus, astrovirus) or follow a specific endemic pattern (rotavirus, enteric adenovirus). Rotavirus is thought to be the infectious agent that most commonly cause severe diarrhea in young children. It is estimated that nearly every child (95%) will have a rotavirus infection before reaching the age of five. There are five species, namely: A, B, C, D and E. Rotavirus A, is the most common cause, being responsible for over 90% of human infections. In temperate countries, rotavirus infections mainly occur during the colder months, whereas in the tropics they can occur throughout the year. Rotavirus is transmitted primarily through the fecal-oral route, from contact with an infected person or a contaminated surface. Improved sanitation is not sufficient to reduce the spread of this virus, as indicated by similar rates of incidence in developed and developing countries. The pathogenic mechanism of rotavirus includes the invasion and destruction of the intestinal villi. The enterotoxin released inhibits the disaccharidase enzymes and glucose-stimulated sodium ion absorption of the microvilli-covered surface of the intestinal epithelium. The illness caused by rotavirus is often severe, can be associated with concomitant fever and vomiting, and is responsible for roughly 40% of all diarrhea-related hospitalizations worldwide. In children between three and thirty-six months of age, the first rotavirus infection is generally the most severe, with subsequent infections being of decreasing severity. Thus, infection likely provides some protection for the host against further severe infections. Diagnosis of infection with rotavirus normally follows diagnosis of gastroenteritis as the cause of severe diarrhea. Most children with gastroenteritis admitted to hospital are tested for rotavirus A. Specific diagnosis of infection with rotavirus A is made by finding the virus in the child's

stool by enzyme immunoassay. There are several licensed test kits on the market which are sensitive, specific and detect all serotypes of rotavirus A. Other methods, such as electron microscopy and PCR, are used in research laboratories (41-45).

Bacteria

Escherichia coli are Gram-negative, rod-shaped bacteria that are commonly found in the lower intestine of warm-blooded organisms (endotherms). Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in humans (intestinal and extra intestinal infections generally severe, such as excretory tract infections, cystitis, meningitis, peritonitis, mastitis, septicemia and Gram-negative pneumonia), and are occasionally responsible for product recalls due to food contamination. The harmless strains are part of the usual flora of the gut, and can benefit their hosts by producing vitamin K2, and by preventing the establishment of pathogenic bacteria within the intestine. *E. coli* and related bacteria constitute about 0.1% of gut flora, and fecal-oral transmission is the major route through which pathogenic strains of the bacterium cause disease. Cells are able to survive outside the body for a limited amount of time, which makes them ideal indicator organisms to test environmental samples for fecal contamination. There is, however, a growing body of research that has examined environmentally persistent *E. coli*, which can survive for extended periods of time outside of the host (46-49). The infectious types are grouped according to factors that characterize their pathogenic mechanism:

- Enterotoxigenic *E. coli* (ETEC) is a common cause of diarrhea in infants and children in developing countries and the most common cause of traveller's diarrhea (50). The infectious dose required for ETEC infection is quite large, and the consequences of its infection include a non-inflammatory diarrhea similar to that of

V. cholera, yet in most cases, less severely dehydrating. The ETEC adhere to the enterocytes, colonize the small intestine, and proceed to secrete toxins. Many clinical isolates secrete just the heat-stable enterotoxin (ST), triggering diarrhea by binding to guanylate cyclase C and causing elevated levels of intracellular cyclic GMP (cGMP). The heatlabile enterotoxin (LT) greatly resembles cholera toxin as it acts on intestinal epithelia, activating adenylate cyclase by ADP ribosylation of GTP-binding protein. ETECs may secrete one or both types of toxin (49-51).

- Enteropathogenic *E. coli* (EPEC) is responsible for thousands of deaths every year, and on average, 5-10% of pediatric diarrheal episodes in the developing world, when diagnosis is made using molecular methods. EPEC adhere to epithelial cells and activate cellular signaling, leading to intestinal secretion. As the bacteria disrupt the microvilli-covered surface of the cell, the absorptive area is diminished. The usual EPEC infection is likely to be significantly longer in duration than other enteric infections. When compared to cases of diarrhea caused by other pathogens, children suffering from an EPEC infection are more likely to develop persistent diarrhea, are more likely to fail to respond to ORS and are more likely to require hospitalization (52, 53).
- Enteroaggregative *E. coli* (EAEC) is an emerging pathogen, and is increasingly recognized as a cause of acute and persistent diarrhea. It can be found all over the world, and both in adults and children. The greatest burden of EAEC is in developing areas, where it is associated with infectious diarrhea in young children and has a tendency to cause persistent illness. EAEC infections most often cause watery diarrhea, albeit it has been suggested that may also have an inflammatory

component. The illness is often indistinguishable from that caused by ETEC. EAEC has been implicated in outbreaks and it is a cause of traveler's diarrhea (54). The bacteria adhere to the epithelial cells and a bio film forms on the surface of the enterocyte, resulting from mucus produced by host and bacteria. Finally, toxins are released, eliciting intestinal secretion and an inflammatory response (48, 54).

- Enterohemorrhagic or verotoxigenic *E. coli* (EHEC): The international pathogens naming convention has recommended the use of STEC (Shiga Toxin Escherichia coli) for this group, because these bacteria produce a toxin cytotoxic to Vero cells growing with structural similarity to the toxin produced by *Shigella dysenteriae*. The STEC produce verotoxins active in the colon causing hemorrhagic colitis and hemolytic uremic syndrome (*Escherichia coli* O157:H7). This strain does not ferment sorbitol and has a phage, where verotoxins are encoded, also called "Shiga toxins". The bacterium has long polar fimbria that is used for adhesion, without strands forming. Paradoxically, treating gastroenteritis secondary to Shiga toxin producing *E. coli* with antibiotics may increase the risk of hemolytic uremic syndrome (55).
- Enteroinvasive *E. coli* (EIEC) is immobile, does not ferment lactose and invades the intestinal epithelium causing bloody diarrhea in children and adults. Calcium is released in large quantities preventing bone solidification and resulting in some cases of arthritis and atherosclerosis. This *E. coli* type cause more damage due to the invasion that occurs in the intestinal epithelium. It tends to occur as occasional outbreaks in developed countries and as endemic infections in developing countries (56).

- Diffusely adherent *E. coli* (DAEC) adheres to the entire surface of epithelial cells and usually causes disease in immunosuppressed or malnourished children and adults. It has been shown that it can cause diarrhea in children over one year of age or in adults and in the elderly (47).

Shigella is the most transcendent pathogen of dysentery. It may be isolated in up to 60% of cases and in almost all severe episodes of this particular diarrhea form. *Shigella* can also cause liquid diarrhea which often precedes dysentery. There are four types: *S. sonnei* (most prevalent in industrialized countries), *S. flexneri* (most prevalent in developing countries), *S. boydii* (rarer) and *S. dysenteriae* (more virulent). The vast majority of infections by *Shigella* bacteria take place in low and middle-income countries (LMIC). Such an infection tends to be more common in young children (up to ten years), but can affect all age-groups. *Shigella* can resist a low pH (acid-resistant), and the small number of organisms required to cause infection can be transmitted *via* contact with an infected person or a contaminated surface. *Shigella* can selectively invade enterocytes as well as M cells, and then multiply and spread inter- and intra-cellularly. The inflammation and ulceration caused by *Shigella* can result in febrile diarrhea or dysentery. The bacteria's secretion of Shiga toxin results in neurotoxic, cytotoxic and enterotoxic effects, blocking the intestine's absorption of electrolytes, glucose, and amino acids. The immune response of the host and generation of cytokines contributes to the disease process, which finally results in necrosis of host cells. Incubation is usually from 24 to 48 hours. The patient develops high fever, severe abdominal cramps, and profuse diarrhea for 24 hours and after this period the diarrhea becomes bloody. The disease lasts for 4 to 5 days. Complications include convulsions, meningitis, pneumonia and sepsis. Due to the severity of diarrhea, antibiotic therapy should always be considered (39, 57, 58).

Vibrio Cholerae is endemic in multiple locations, including Africa and Asia, and is a cause of large-scale outbreaks. This bacterium has 139 serotypes based on their antigenic profiles. The biotype 01 has disseminated the disease since 1960; biotype El Tor has been responsible for most recent outbreaks. The other non-01 types cause sporadic episodes. Transmission is fecal-oral by contaminated food and water and has been responsible for several highly infective outbreaks. The incubation is in general from a few hours to five days. Untreated cases have a high case-fatality rate. *V. cholerae* are easily destroyed by the gastric acid, and millions of organisms are required for symptomatic infection. Consequently, this microbe must first multiply in food or water in order to reach a sufficient number to cause infection. A toxin released by the bacterium (the cholera toxin, CTX) is responsible for the massive, watery diarrhea characteristic of cholera infection. With no histological changes in the intestine, many cases are asymptomatic while others develop profuse watery severe diarrhea, with significant loss of water and electrolytic. The feces are "rice water" type and the patient should immediately receive hydration proactively. Death usually occurs in relation to the massive dehydration and not on account of the infection *per se*. Several vaccines have been developed, but none are yet available for large scale use (1, 46, 59-62).

Salmonella, a gram-negative bacillus can be divided into typhoid (*S. typhi*) and non-typhoid (NTS). Among the non-typhoid, the predominant is *S. enteritidis* with various serotypes. Non-typhoid *Salmonella* serovars cause as much as an estimated 1 billion cases of gastroenteritis in humans every year. *Salmonella* undermine cellular signaling, membrane trafficking and pro-inflammatory responses. Upon ingestion, *Salmonella* invade the intestinal mucosa by multiple mechanisms, including the invasion of M cells and their active uptake by dendritic cells, and they replicate intra-cellularly in non-phagocytic cells. The pathogen can induce cell death in various types of host cells. *Salmonella*-induced

gastroenteritis can present with diarrhea and concurrent fever. Typhoidal *S. enterica* serovars, mostly restricted to humans, are responsible for some 20 million cases of enteric fever (also known as typhoid fever) worldwide every year. While most *Salmonella* infections remain localized to the intestine and cause diarrhea, typhoid strains can survive in intestinal macrophages, disseminate to the liver and spleen and cause a potentially life-threatening systemic infection. The incubation period of typhoid fever is usually of 7 to 14 days (range established 3-60 days). In non-typhoid *Salmonella* infections, antibiotics may increase the risk of prolonged carriage and disease relapse (63-67).

Campylobacter jejuni is a species of curved, helical-shaped, non-spore forming, Gram-negative, microaerophilic bacterium commonly found in pet feces. It is one of the most common causes of human gastroenteritis in the world. Food poisoning caused by *Campylobacter* species can be severely debilitating, but is rarely life-threatening. It has been linked with subsequent development of Guillain-Barré syndrome, which develops usually two to three weeks after the initial illness (68).

Aeromonas are gram-negative bacilli that can determine gastroenteritis in children; especially those under three years of age after the ingestion of contaminated water or food. Diarrhea is typically watery with accompanying fever and abdominal pain. Acute diarrheal disease is self limited, and only supportive care is indicated in affected patients. Severe *Aeromonas* gastroenteritis resembles shigellosis; with blood and leukocytes in the stool and antimicrobial therapy is necessary for patients with systemic infection or chronic diarrheal disease. Although some potential virulence factors (e.g. endotoxines, hemolysins, enterotoxins, adherence factors) have been identified, their precise role is unknown (69).

Clostridium difficile is the cause of an antibiotic-associated diarrhea and secondary to its toxin, commonly known as pseudomembranous colitis. This organism produces spores that are spread from person to person. The diarrhea associated to *C. difficile* can occur after exposure to any antibiotic, but is classically associated with clindamycin and/or to quinolones (70).

Yersinia enterocolitis is also a gram-negative bacterium that causes gastroenteritis in children and adults, especially in temperate countries. It seems to be more related with eating pork and contaminated milk. The symptoms include cramps, vomiting, fever and watery or bloody diarrhea and fever. The presentation may last 7-21 days, although the pathogen may be detectable for weeks, even after the clinical picture has improved (49).

Staphylococcus aureus is a facultative anaerobic Gram-positive coccal bacterium frequently found in the human respiratory tract and on the skin. *S. aureus* is not always pathogenic but may cause skin infections, respiratory disease and food poisoning. Staphylococcal food poisoning is an intoxication which typically occurs after ingestion of different foods, particularly processed meat and dairy products containing sufficient amounts of *S. aureus* with subsequent enterotoxin production by improper handling and storage at elevated temperatures. Symptoms of food poisoning have a rapid onset (2–8 h), and include nausea, violent vomiting, and abdominal cramping with or without diarrhea. The disease is usually self-limiting and typically resolves within 24–48 h after onset. Occasionally it can be severe enough to warrant hospitalization, particularly when infants or elderly are concerned. Antibiotics are not useful in treating this illness. The toxin is not affected by antibiotics.

Parasites

Both within the small and large intestine live a wide variety of infecting parasites, including nematodes, cestodes, trematodes and protozoa. These intestinal parasites can act in different ways: damaging directly the mucosa, interacting with the host immune system and competing with the nutrients. Many are the parasitic infections that can debut with a clinical episode of diarrhea. However, most common causative agents of diarrhea include, among others: *Giardia lamblia*, *Entamoeba histolytica*, *Balantidium coli*, *Cryptosporidium parvum* and *Strongyloides stercoralis*.

In the majority (60%) of patients infected with *G. lamblia* parasite, infestation is asymptomatic. However Giardiasis usually presents as chronic diarrhea type of malabsorption, which can also occur with diarrhea secondary to *Strongyloides stercoralis*.

Amebiasis may be asymptomatic, or determine dysentery episodes with profuse diarrhea accompanied by blood and rectal tenesmus and even extra-intestinal complications (amebic abscesses in the liver, lung and brain).

Cryptosporidium are common among children in developing countries; frequently asymptomatic, or may cause mild watery diarrhea in immunocompetent patients, which solves without treatment. It may, however, lead to prolonged and severe diarrhea in HIV patients and other immunocompromised hosts.

Other diarrheal disease causes

Diarrhea may also result from other specific infections (malaria, pneumonia etc.) or arise within the context of a non-communicable disease. These non-infectious causes of diarrhea are very varied, and include, among others, intolerance to carbohydrates, proteins, gluten or hyperosmolar diets; anatomical and mechanical problems (short bowel) or autoimmune or rheumatic diseases (Crohn's disease, ulcerative colitis, Whipple's disease, necrotizing

enterocolitis, pseudomembranous colitis, Hirschsprung Disease, intussusception); biochemical (abetalipoproteinemia, chylomicron retention, chloridorrheic congenital acrodermatitis enteropathica, scleroderma, diabetes), drugs (particularly post-antibiotic treatment); pancreatic and liver diseases (biliary atresia, cirrhosis, chronic pancreatitis, cystic fibrosis); endocrine diseases (hyperthyroidism, congenital adrenal hyperplasia, Addison's disease, hypoparathyroidism); neoplasms (carcinoid, ganglioneuroma, neuroblastoma, polyps, lymphoma, mastocytosis, adenocarcinoma) and toxics (71).

4.1.4 Physiopathology of diarrheal disease

The normal balance of intestinal fluid

The total amount of liquid to be absorbed by the intestine is equal to daily liquid intake (about 1.5 liters) plus the amount secreted in several gastrointestinal secretions (about 7 liters). This represents a total of 8 to 9 liters. All this liquid, except for approximately 1.5 liters, is absorbed by the small intestine, so that only 1.5 liters daily pass through the ileocecal valve into the colon. Of these, only 100ml daily are excreted with the feces, the remainder being absorbed. During this process, water and electrolytes are simultaneously absorbed by villi and secreted by crypts of the epithelium of the small intestine. This causes a reverse flow of water and electrolytes across the intestinal lumen and the blood. Since usually the absorption of fluid is greater than its secretion, the result is of an overall fluid absorption. Any change of the bi-directional flow of water and electrolytes into the small intestine (ie, increased secretion, decreased absorption or both) results in sharp absorption and causes an increase in the volume of liquid entering the large intestine. Diarrhea occurs when the volume exceeds the limited absorption capacity of the large intestine.

Intestinal absorption and secretion of water and electrolytes

The water absorption from the small intestine is caused by osmotic gradients that are created when the solutes (particularly sodium) are actively absorbed from the gut lumen by the villi of the epithelial cells (60, 71). There are several mechanisms by which sodium is absorbed in the small intestine: sodium connected with uptake of chloride ion, directly absorbed as sodium ion, exchanged for hydrogen ion or connected to the absorption of organic substances such as glucose or certain amino acids.

The secretion of water and electrolytes normally occurs in the crypt of epithelium of the small intestine, where sodium chloride is transported from extracellular fluid to epithelial cells through the basolateral membrane. Sodium is then pumped back to the extracellular fluid by the NaK-ATPase. Simultaneously, the secretor stimulus causes the passage of chloride ions across the luminal membrane of the cells of the crypts into the lumen of the intestine. This creates an osmotic gradient, which causes the passive flow of water and other electrolytes into the lumen of the intestine through intercellular channels (71).

Mechanisms of diarrhea

Secretory diarrhea

This occurs when absorption of sodium in the villi is impaired while the secretion of chloride in the crypt cells continues to increase. This situation occurs in infection with cholera and Enterotoxigenic *E. coli* or food poisoning in which case the toxins from the microorganisms stimulate secretion of fluid.

Invasive diarrhea

This occurs when there is disruption of the intestinal mucosal cell as a result of invasion by bacteria (*shigella*, *C. jejuni*, *enteroinvasiva E.coli* and *salmonella*) or protozoa (*E. hystolitica*).

Motility diarrhea

This occurs due to increased motility (peristaltic action) of the gastrointestinal tract resulting in decreased in the transit time of food or drink across the gastrointestinal tract. This gives less chance for the contents to be absorbed. Examples of such a kind of diarrhea include that secondary to thyrotoxicosis, hyperkalemia or the use of purgatives.

Osmotic diarrhea

This occurs due to decreased absorption of osmotically active substances, which draw fluids in the gastrointestinal tract with them by osmosis and make the stool become looser or watery. Lactose intolerance and laxatives are an example of this kind of diarrhea.

Malabsorption syndrome

This occurs because of decreased absorption of nutrients as a result of abnormality in the absorptive surface area (example sprue, gluten induced enteropathy, steatorrhea, enzyme deficiencies, etc).

4.1.5 Clinical presentation associated with diarrhea

Table 1 presents a widely accepted clinical classification of diarrheal diseases, including etiology and pathogenesis. Based on the duration and stool characteristics, diarrhea may be sub-classified in acute diarrhea, dysentery, persistent, and chronic diarrhea. Acute diarrhea

refers to episodes lasting less than 7 days. Dysentery is defined as the passage of blood and

Table 1: Etiology of diarrhea (table from Mathan, *British Medical Bulletin* 1998)

Acute infectious diarrhea
<p>Watery diarrhea</p> <hr/> <p>Enterotoxin associated- CTX, LT, ST, ZOT</p> <p>Enteroadhesive associated: aggregative, adherent <i>E. coli</i></p> <p>Cytotoxin associated: EPEC, Shiga-like toxin, etc.</p> <p>Viral diarrheas: rotavirus, adenovirus, Norwalk virus, etc.</p> <p>Parasite associated: Giardia, <i>Cryptosporidium</i>, Isospora</p> <p>Unknown mechanism: anaerobes, Giardia</p> <p>Dysentery</p> <hr/> <p>Invasive bacteria: <i>Shigellae</i>, <i>Salmonellae</i>, <i>Campylobacter</i></p> <p>Parasites: <i>E. histolytica</i></p> <p>Mucoid diarrhea: any of the pathogens which cause watery diarrhea or dysentery</p> <p>Antibiotic-associated diarrhea: <i>Clostridium difficile</i></p> <p>Parenteral diarrhea</p> <p>Traveller's diarrhea</p>
Persistent diarrhea
<p>Chronic diarrhea</p> <p>Malabsorption syndromes</p> <hr/> <p>Secondary malabsorption syndromes</p> <p style="padding-left: 100px;">Luminal factors</p> <p style="padding-left: 100px;">Mucosal factors</p> <p style="padding-left: 50px;">Interference with vascular and lymphatic transport</p> <p style="padding-left: 50px;">Pancreatic and biliary deficiency</p> <hr/> <p>Primary malabsorption syndrome: tropical sprue</p> <p>Inflammatory bowel diseases</p> <p>Diarrhea of the Immunocompromised</p> <p>Irritable bowel syndrome</p>

mucus in the stools (72). Persistent diarrhea occurs when the duration exceeds seven days and chronic diarrhea when it lasts more than 14 days. Gastroenteritis refers to a syndrome characterized by the presence of gastrointestinal symptoms, including nausea, vomiting, diarrhea and abdominal discomfort. Enterocolitis is the inflammation of the mucosa of the small and large intestine.

One can also classify it into “high” and “low”, according to the segment of the intestinal tract affected. Diarrhea is high when there is involvement of the small intestine, characterized by few stools, high volume, and the presence of certain food debris (Rotavirus, *Salmonella*, *V. cholera*, *Giardia*, *Cryptosporidium*). “Low” Diarrhea is that which translates low involvement of the large intestine, with large number of stools, with tenesmus, and there may be presence of blood and pus (*Shigella*, *E. coli*, *Enteroviruses*, *C. jejuni*, *E. histolytica*). Diarrhea may also affect both upper and lower segments of the intestine, and is thus termed “mixed”.

4.1.6. Diagnosis of diarrheal infection

The bacteriological examination of stools is recommended in patients with fever, profuse diarrhea with severe dehydration, malnutrition, immunodeficiency, in cases of suspected hemolytic uremic syndrome or during outbreaks (72). To diagnose the infection, a series of tests may be run, including stool tests (gram-stain, ova and parasites investigation, fecal leukocytes, culture and toxin and antigen assay) and/or blood tests (serology and culture).

4.1.7 Dehydration and other complications

The principal complication of any diarrhea episode is dehydration. Other complications include damaging effects on other body systems and reduced appetite, change in feeding practices and in the absorption of nutrients which to the most extreme cases can lead to

malnutrition (discussed in Section 4.2) usually in the context of diarrheal events that become persistent and subsequently chronic. Repeated diarrheal episodes or those with associated complications may lead to diminished growth and impaired cognitive development, particularly in resource-limited countries.

Dehydration secondary to increased loss of body volume (water and electrolytes such as sodium, chloride, potassium and bicarbonate) in relation to loose stools and vomiting without proper and adequate water and electrolyte replacement represents the greatest danger of diarrheal episodes. Although the early stages of dehydration present no symptoms or signs, as dehydration progresses, symptoms can be severe and progress to shock, a potentially fatal complication. Patients with diarrhea and dehydration must be evaluated to establish the degree of dehydration, which is evidenced by the presence of certain signs and symptoms related to ongoing losses of body fluids (73).

The degree of dehydration is rated, according to WHO (74) on a scale ranging from mild to moderate or severe:

- *Mild dehydration* (3-5% fluid losses), characterized by normal pulse or minimum tachycardia, thirst and the rest of the physical examination remaining normal.
- *Moderate dehydration* (6-10%) characterized by tachycardia, low urine output, irritability or lethargy, sunken eyes, depressed anterior fontanelle, decreased tear emission, dry mucous membranes, decreased skin elasticity, slowing time capillary refill (≤ 2 seconds), cool pale skin.
- *Severe dehydration* (10-15%) characterized by tachycardia with weak pulse, hypotension, pulse *tardus*, significant reduction or even interruption of urinary output, sunken eyes, very depressed fontanelle, no tears, very dry mucous

membranes, marked decrease in skin elasticity, increase in the capillary refill time (\geq 3 seconds) with very cold skin.

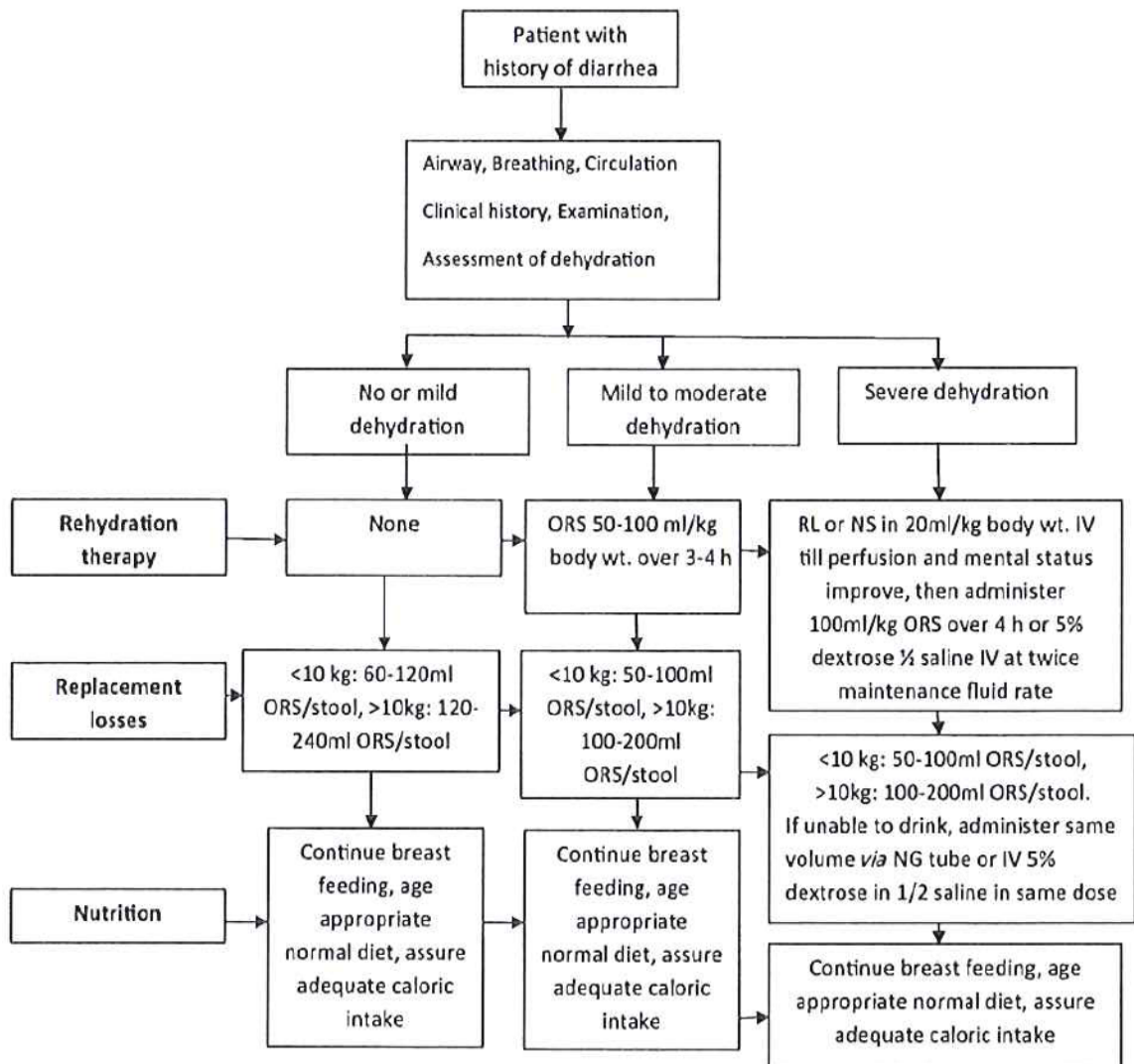


Figure 2: Simplified algorithm for rehydration therapy of acute diarrhea (figure from Dekate et al, Indian J Pediatr 2012)

If the child is not hydrated promptly, death can occur very quickly. Thus dehydration can be prevented by rapidly and adequately replacing the fluids and electrolytes lost. The degree of dehydration gives the urgency and the amount of liquid needed for rehydration. Mild to moderate dehydration is often treated with oral hydration solutions with low osmolarity

(200-250 mOsm/l) and sodium (60-70 mmol/l) that contain glucose, potassium and a base such as citrate. Severe dehydration usually requires urgent intravenous hydration and may require urgent hospitalization. Figure 2 presents clinical management according to dehydration degree. Malnourished children with dehydration should be treated with reduced Osmolarity ORS (resomal) and extra care should be taken with electrolyte replacement, and particularly with potassium supplements. Dehydration should be corrected slowly over 12 hours and resomal should be given at 10 ml/kg/h for first two hours followed by at 5–10 ml/kg every hour for next 4–10 h (by oral/nasogastric tube), adjusting the intake to the child's capability to drink, volume of fluid loss in stools and vomiting. The only indication for IV infusion in a severely malnourished child is circulatory collapse caused by severe dehydration or septic shock. Careful frequent reassessment is needed for signs of rehydration and over hydration (over hydration in a malnourished child is dangerous due to the high risk of heart failure because of poor cardiac reserve and pumping capability).

4.1.8 Useful measures to combat diarrhea: preventive and curative tools

Exclusive breastfeeding for the first six months of life, and held in conjunction with the correct introduction of other foods until the second year of age; vitamin A supplementation, hand hygienic practices, access to safe drinking-water, use of facilities for sanitations and control measures against flies, fluid replacement to prevent dehydration with oral rehydration salts and implementing proper nutritional habits including continuous feeding during the acute diarrheal can all contribute to lessen the impact of diarrhea and are the basis for disease control (75, 76). Other disease control measures include health education about how infection spreads and use of healthcare facilities (17). Recently, WHO and UNICEF have recommended the use of zinc supplements for the treatment of diarrhea, because there is evidence that their use reduces the duration and severity of the acute episode (77).

Supplementations of probiotics could potentially also reduce severity and duration of diarrhea; however evidence does not support yet their routine use or that of antisecretory, antimotility and binding agents (72). Existing vaccines for diarrhea are scarce in the developing countries and include the measles vaccine (78, 79), whose action against diarrhea is indirect, but whose implementation with high coverage could prevent up to an estimated 3-8% of episodes of diarrhea and 6-26% of diarrhea-associated deaths in children under 5 years. Also noteworthy is the cholera vaccine (80, 81), with an acceptable efficacy but hampered by the duration of its protection, limited in time. The new rotavirus vaccines, recently introduced in the market, have proven to be safe and effective (82). However, their coverage in low income countries is still anecdotal, showing much room for improvement. Also, there are two typhoid vaccines (83) currently approved for clinical use, albeit none of them is suitable for distribution to children in developing countries.

The use of antibiotics to treat diarrheal acute episodes is only recommended in particular situations (for bacterial gastroenteritis complicated by septicemia and in cholera, shigellosis, amebiasis, giardiasis, *E. coli* enteroinvasive and enteric fever, in severely malnourished children, newborn babies and young infants with less than 3 months of age and in immunocompromised patients (HIV infection, lymphoreticular malignancy, receiving chemotherapy, having undergone organ transplantation)), and their effectiveness is often questioned, because of its associated risk of fueling antibiotic resistance. For any of the abovementioned circumstances, the recommended antibiotics and respective doses include (74):

1. *Diarrhea with clinical signs of sepsis*: (toxic appearance, leukocytosis, fever $> 38.5^{\circ}$ C, septic shock): Ceftriaxone 50 –100 mg/kg/d IV/IM divided 12 hourly for 7–10 days.

2. *Diarrhea in a child with severe malnutrition*: Ampicillin 200 mg/kg/d IV/IM divided 6 hourly along with Gentamicin 5 mg/kg/d IV/IM 8 hourly for 7–10 days.
3. *Newborns and very young infants (< 3 months) with fever (> 38.5°C)*: Cefotaxime 150 mg/kg/d IV/IM divided 8 hourly.
4. *Dysentery (bloody stools) and diarrhea during outbreak of shigellosis*: Ceftriaxone IV/IM 50–100 mg/kg/d for 7 days or Ciprofloxacin orally 20–30 mg/kg/d divided 12 hourly for 7–10 days.
5. *Suspected Cholera* ('Rice water stools' with high purge rate i.e., > 10 large volume stools/d): Doxycycline 300 mg single dose or ciprofloxacin 1g single dose; or Azithromycin 20 mg/kg single dose. In children or pregnant women, an acceptable alternative includes the use of erythromycin 500mg/8h, 3 days. Treatment of cholera decreases duration of disease and mortality, and controls the transmission.
6. *Suspected amebiasis or giardiasis* (colitic stools, anorexia and weight loss, persistent diarrhea, failure to thrive): Metronidazole 30–40 mg/kg/day, p.o, divided 8 hourly for 7–10 days
7. *Cryptosporidium*: Nitazoxanide 500mg/12h/p.o., for three days. In children >1 year of age 100mg/12h/3 days. In HIV positive patients it is critical to continue or start antiretroviral therapy concomitantly.

4.2 Malnutrition

4.2.1. Brief introduction

Malnutrition can be defined as an imbalance between external food intake and the body's requirements. It can be of the acute type (wasting; based on a decreased weight-for-height), chronic type (stunting; based on a decreased height-for-age) and mixed type (underweight based on a decreased weight-for-age) (84). WHO/UNICEF recommend the use of a cut-off for weight-for-height of below -3 standard deviations (SD) of the WHO standards or/and the presence of edema to identify infants and children as having severe acute malnutrition (SAM). Children aged 6-60 months with a MUAC less than 115 mm have a highly elevated risk of death compared to those who are above that specific threshold. Thus, WHO also recommended to increase the cut-off point from 110 to 115 mm to define SAM with MUAC (85).

Globally, malnutrition is associated directly or indirectly with at least a third of all childhood mortality in developing countries, with the risk of mortality being 4 folds higher among severely compared to moderately malnourished children (9). Case-fatality rates in hospitals treating SAM in developing countries average 20-30% and have remained unchanged since the 1950's despite the fact that clinical management protocols capable of reducing case-fatality rates to 1-5% have been in existence for over 30 years (86). Significantly, Sub-Saharan Africa and southern Asia carries the brunt of the impact caused by malnutrition, as almost half of the associated deaths occur there (Table 2) (87, 88). Factors contributing to the high mortality in children with malnutrition include the appearance of infections (eg. Diarrhea, pneumonia etc.), low socioeconomic status of the family and its related consequences such as unavailability of appropriate nutritional foods and poor access to health services associated to the lack of good clinical skill.

Table 2: Levels and trend of child malnutrition in 2011 (table modified from WHO/UNICEF, The World Bank Joint Child Malnutrition Estimates, 2012)

	Burden of Malnutrition	
Stunting	Globally, an estimated 165 million children under-five years of age, or 26% were stunted (i.e, height-for-age below -2 SD) in 2011, a 35% decrease from an estimated 253 million in 1990.	High prevalence levels of stunting among children under-five years of age in Africa (36% in 2011) and Asia (27% in 2011) remain a public health problem, one which often goes unrecognized.
Underweight	Globally, an estimated 101 million children under-five years of age, or 16% were underweight (i.e., weight-for-age below -2 SD) in 2011, a 36% decrease from an estimated 159 million in 1990	Although the prevalences of stunting and underweight among children under-five years of age worldwide have decreased since 1990, overall progress is insufficient and millions of children remain at risk.
Wasting	Globally, an estimated 52 million children under-five years of age, or 8%, were wasted (i.e., weight-for-height below -2 SD) in 2011, a 11% decrease from an estimated 58 million in 1990.	Seventy percent of the world's wasted children live in Asia, most in South-Central Asia. These children are at substantial increased risk of severe acute malnutrition and death.

4.2.2 Physiopathology and clinical features

The insufficient supply of protein, carbohydrates and fat is the major cause of protein-energy malnutrition. Then appear severe and chronic infections, particularly those producing diarrhea, but also other infections such as helminthic infections. The underlying mechanisms include decreased food intake because of anorexia, decreased nutrient absorption, increased metabolic requirements and direct nutrient losses (89).

The pathologic changes include immunologic deficiency in the humoral and cellular subsystems arising from protein deficiency and lack of immune mediators (e.g., tumour necrosis factor). Metabolic disturbances also play a role in impaired intercellular degradation of fatty acids because of carbohydrate deficiency. Synthesis of pigments in the hair and skin fails (e.g., hair color may change and skin become hyperpigmented) because of a lack of substrate (e.g., tyrosin) and coenzymes.



Figure 3: Children with kwashiorkor and marasmus (Photos by Quique Bassat©)

Two very distinct but sometimes overlapping clinical syndromes of malnutrition have been classically described (figure 3). They both arise from a different pathophysiological

pathway. Marasmus, which appears on account of a severe and prolonged decreased caloric intake, is diagnosed when subcutaneous fat and muscle are lost because of endogenous mobilization of all available energy and nutrients. Clinical aspects typically include a triangular face, extended abdomen (from muscular hypotonia) and anal or rectal prolapse (from loss of perianal fat).

Kwashiorkor, resulting from specific protein-deficient diets, usually manifests with edema, changes to hair and skin color, anemia, hepatomegaly, lethargy, severe immune deficiency and early death. Weight loss may not be such a differentiating issue in such cases because of the generalized liquid retention. Despite decades of debate, sometimes quite intense, the pathologic features of kwashiorkor are still not fully understood (89). The role of aflatoxins and insufficient protein intake has been stressed because the presence of edema and ascites seems related to reduced osmolarity in the blood, which is thought to be caused mostly by severe anemia. It is puzzling that total protein concentrations in the plasma do not differ between children with marasmus and those with kwashiorkor. More recently, a role for free radicals in the etiology of kwashiorkor has been considered, but the findings of initial intervention studies have not been up to expectations (89). This may possibly be the result of inappropriate experimental design.

One essential aspect of severe protein–energy malnutrition is the fatty degeneration of such diverse organs as the liver and heart. This degeneration is not just a sign of severe malnutrition; it causes subclinical or overt cardiac insufficiency, especially when malnutrition is accompanied by edema. If the myocardial insufficiency is not corrected, iatrogenic fluid and sodium overload quickly escalate it into cardiac failure. A second injurious aspect is the loss of subcutaneous fat, which markedly reduces the body's capacity for temperature regulation and water storage. As a consequence, malnourished children become dehydrated, hypothermic and hypoglycemic more quickly and severely than others.

Finally, severe protein-energy malnutrition is associated with atrophy of the mucosa of the small bowel, leading to a loss of absorption as well as of digestion capacity (89).

Severe malnutrition is furthermore associated with chronic hypovolemia, which leads to secondary hyperaldosteronism, and further complicates fluid and electrolyte balance. Because the development of muscular dystrophy mobilizes much of the body's potassium, which is then lost through urinary excretion, affected children do not show signs of hyperkalemia. Most children with severe protein-energy malnutrition have asymptomatic infections because their immune system fails to respond with chemotaxis, opsonization and phagocytosis of bacteria, viruses or fungi. So depressed is the system that the body cannot produce even the fever that is typical of inflammation. Not only do protein-energy malnutrition and micronutrient deficiencies overlap, but a lack of one micronutrient is typically associated with deficiencies of other micronutrients including iron, iodine, vitamin A and zinc (90).

4.2.3 Malnutrition management

The WHO has proposed standard guidelines on the diagnosis and treatment of malnutrition in order to reduce the risk of morbidity and mortality, shorter hospital stay and facilitate rehabilitation and full recovery (86, 91-93). Severe wasting children should all be admitted. However, children < 60% weight-for-age may be stunted, and not severely wasted. Stunted children do not require hospital admission unless they have a serious concomitant illness.

The treatment of children with severe malnutrition is divided into three phases, namely:

1. Initial treatment that includes identifying and treating problems that endanger life in a hospital or clinic (hypothermia, hypoglycemia, dehydration, infection): Clinicians should promptly correct the specific deficiencies, detected metabolic abnormalities and cautiously start feeding.

2. Rehabilitation: in this stage, intensive feeding is administered to recover most of the lost weight, micronutrient deficiency supplementation and deworming. Emotional and physical stimulation should also be provided. The mother or the person responsible for care is trained to continue care at home and preparations are made for discharge of the child.
3. Follow-up: This corresponds to the stage after discharge in which an adequate control of the child and family to prevent relapse and ensure the physical, mental and emotional progressive child should be put in place.

Successful treatment of children with severe malnutrition does not require any sophisticated facilities and equipment or highly qualified personnel. However, it requires treating every child with proper care and affection and that each phase of treatment is carried out properly by health professionals with a dedication and proper training. When this is done, the risk of death can be reduced significantly and the chances of a full recovery are increased. Though, if considering that the disease is only a medical disorder, it is likely that the child relapses when at home and that other children in the family remain at risk of suffering the same problem. Thus, adequate treatment of severely malnourished child requires identifying and correcting also social problems.

4.3 Health care access and utilization

Health centers are the base of the health system, assuming the role of clinical assistance based on socially-acceptable care and scientifically proven technology, and with the ambition to ensure universal access to individuals and families. It is the gateway to individual care, responsible for the ongoing monitoring of users and is in a better position to interpret and contextualize their health problems to the social environment in which they live. The use of health services is the result of a process in which the individual's need and his decision to seek medical and hospital care are met(94). This process depends on a number of factors that can be schematically divided in four groups of explanatory determinants of health utilization: 1) the perceived need; 2) the predisposing determinants (age, sex, household size and education/culture); 3) the enabling determinants (location, access roads, public transport and economic status) (95, 96); and finally 4) the health services system determinants (97).

4.4 Diarrheal disease, malnutrition and healthcare utilization in Mozambique

4.4.1 Diarrheal disease and malnutrition in Mozambique

Mozambique is one of the poorest sub-Saharan African countries, with an under-five mortality rate of 135 deaths per 1000 live births, estimated in the year 2010 (98). Data from the Mozambican Ministry of health (MISAU) indicates that diarrheal diseases remain one of the leading causes of illness and death among children under 5 years in Mozambique (99). Indeed, diarrhea is among the most common presentation to health care facilities and during the last years the number of cases in children aged less than 5 years has steadily increased through the country from 120,000 in the year 2000 to 240,000 by the end of the decade (100). However, the accuracy of the MISAU data are heavily dependent on access to health facilities and pattern of health seeking behavior, and need to be taken with certain caution, as it may include important bias, particularly in relation to access. In this respect, it has been estimated that only 35-40% of the population receives some curative facilities from the National Health System, which means that >60% of the population have important access constrains. In such cases, epidemiological studies become necessary for obtaining reliable data to guide the planning and conduct of control strategies, as the silent burden of diarrhea is greatest in those rural areas with a potentially highest burden in relation to a higher presence of the commonest risk factors. The few epidemiological studies realized in this country indicate that diarrhea is estimated to be the third leading cause of death (accounting for at least 10% of all mortality) among children aged 0-14 years in the city of Maputo, the capital and an urban environment (101). In the district of Manhiça, predominantly rural, diarrhea is the third leading cause of hospital admission among children aged 0-14 years and the fourth leading cause of death among children between 12 and 59 months, according to verbal autopsies performed in the area (102). In another study, pediatric diarrheal disease

was estimated to account for over 13,000 annual deaths, *circa* 7-12% of the 110,420 estimated annual Mozambican under five deaths (9).

In Mozambique, as usually occurs in most other of sub-Saharan African a multitude of factors contribute to the high diarrheal disease burden, especially among younger children. Beyond the poor access to health facilities, the illiteracy rate reaches 53.5% of the population. Index female illiteracy is higher than male illiteracy: 71.3% vs. 43% and higher in rural areas. Consequently, although the rate of breastfeeding is high, low maternal education does not allow adequate dietary practices especially during the weaning or compliance to prevention of mother to child AIDS transmission measures, highly prevalent in Mozambique. In this country, 44% of children under the age of five are stunted due to chronic illness and poor diet (103). Around 18% of children are underweight, with children living in rural areas being almost twice as underweight as those living in towns and cities (103). The prevalence of wasting is less than 5%. Malnutrition (weight-for-age) is the fourth cause of hospital admission in the pediatric wards and is associated with high case fatality rates (CFR), reaching 20% in Maputo's central hospital; the national referral hospital located in an urban area (104). In the district of Manhiça, malnutrition is the fourth leading cause of hospitalization and third cause of death according to reports from the hospital and verbal autopsies (102). Measles immunization that is recognized to substantially reduce the incidence and severity of diarrheal diseases is the only currently available vaccine in Mozambique that may prevent diarrhea, but its estimated coverage is very high (97%) (98).

While considerable progress has been made over the past years to bring water supply and sanitation to more people, water and sanitation remains one of Mozambique's most under-developed areas. According to the latest data available, only 43% of the population has access to safe water and 19% of the population has access to improved sanitation. The

situation in rural areas is far worse than that of urban areas with only 30% of the rural areas having access to water and a mere 6% having access to safe sanitation (105).

A marked seasonality characterizes diarrhea in Mozambique, which tends to occur more frequently during the rainy season with frequently occurrence of cholera outbreaks (firstly reported in 1959). In Mozambique cholera began to pose a health problem in 1983, since then the country has suffered cholera epidemics consecutive (100). In recent years, WHO has proposed a change from “epidemic to an endemic disease”, due to the cumulative number of asymptomatic at the end of each peak associated to continuously emerging tendency *V cholerae* resistance to antibiotics.

4.4.2 Healthcare in Mozambique

Health care and the right to health care are recognized in the Constitution of Mozambique (Article 94), however according to MISAU, health indicators are very low. In this country, an estimated 65-70% of population lives in rural areas and people who live in rural areas are disadvantaged in terms of health in several ways compared with their urban counterparts. These disadvantages include limited access to health care as a result of geographic barriers, such as time and distance to care sites, and availability of transportation. Thus, up to 60% of the Mozambican population has no access to curative facilities, as reported from the National Health Service (NHS), when defining this inaccessibility as living 20 km or further away from any health facility.

The NHS covers primarily the urban and peri-urban areas and is heavily dominated by the public sector as the major provider in the country. The for-profit sector is largely confined to major cities, and virtually non-existent in majorly rural areas. Many international and national Non-governmental organizations (NGOs) and Faith-based organizations (FBOs) operate mainly at the district level and offer a range of preventive and curative services.

Networks of community health workers (CHWs), most of who serve on a voluntary basis, are limited in size and distribution. In rural areas, traditional healers and herbalists provide the first link in the chain of access to health care and referral in the country.

The National Health System in Mozambique is managed at three levels: 1) Ministry of Health (with four offices: National health direction, Planning and Cooperation direction, Human Resource direction and Administration and Management direction); 2) Provincial Health direction; and 3) District Health direction. It is organized into four service delivery levels (Table 2):

- Level I, which include health posts and health centers. These infrastructures are able to offer basic diagnostic services, including microscopy, blood counts, biochemistry and X-rays, while health centers with limited capacity may only offer medical admission with medical and non-surgical obstetric conditions. In this level, health center facilities are staffed with general medical doctors while in posts health, care is provided by clinical officers, nurses, and medical technicians; however most health facilities are understaffed.
- Level II, includes rural/district hospitals and general hospitals which beyond the abovementioned facilities provide basic surgical and obstetric conditions.
- Level III, which include provincial hospitals and provide greater diagnostic and curative services, and include training centers for provincial health care staff.
- Level IV includes central and specialized hospitals. There are only three central hospitals in the entire country.

Despite improvements in recent years, the health situation in Mozambique remains particularly worrying. The facilities often have limited supplies and drugs, lack suitable sources of water and are staffed by overstretched health workers with insufficient

training. It is necessary to strengthen the training of health personnel and promote gender equity. In Mozambique there is one doctor for every 22,000-25,000 inhabitants. The expanded immunization programme (EPI) currently includes the following vaccines: Tuberculosis-BCG, Diphtheria, Pertussis, Tetanus, Polio, Viral Hepatitis B, Measles, *Haemophilus influenzae* b conjugate vaccine (since 2010) and recently introduced (April 10th, 2013) anti-Pneumococcal conjugate vaccine.

Table 3: The health network organization in Mozambique (MISAU report)

Level	Category of Health Unit	Aproximate number in operation	Aproximate number in beds	Type of care provided
I	Health Post	700	7200	Primary (preventive and curative) care
	Health Centres	300		
II	Rural/districtal	30	3200	"First reference", with services of admission and basic surgery
	Hospitals General Hospitals			
III	Provincial Hospitals	7	1800	Surgery, obstetrics, gynaecology, paediatrics, internal medicines, orthopaedics and stomatology
IV	Central Hospital (CH)	3	2900	The most differentiated HU with multiple specialties (above all in Maputo CH, that has about half of the beds at this level)
	Psychiatric Hospitals	2		
Total		1414		

5. SPECIFIC INTRODUCTION TO THIS THESIS

The burden of diarrheal disease and malnutrition is still very high in the world, but particularly distressing in developing countries. Thus, there is a need to improve strategies that may diminish morbidity and mortality from diarrheal disease and malnutrition. Since these two diseases are bi-directionally related and as well some of the risk factors like poor access to health services and the worst social conditions appear to be commonly shared, one might assume that strategies to combat one of these diseases will have an implication also in the other.

Thus, with the purpose of guiding public health policies and target appropriate interventions; there is a compelling need to determine the etiology, burden and *sequelae* of diarrheal disease (including malnutrition) in settings where diarrheal diseases remain a major contributor to child mortality. These data must be produced using robust methodologies that can subsequently inform on the most adequate strategies to diminish morbidity and mortality from diarrheal and malnutrition diseases, with a clear emphasis on children living in regions where mortality is high, such as those in sub-Saharan Africa. The scope of the work that is the basis for this thesis is to respond to such necessity with complementary data that will provide an overall adequate picture of the current situation of diarrheal and malnutrition disease in a Mozambican rural area, ranging from the basic social investigation of the determinants of use of health services in such cases, to the most specific analyses of clinical and microbiological determinants of the disease.

This thesis is presented as a collection of five articles describing the work undertaken through a partnership between the *Centro de Investigação em Saúde da Manhiça* in Mozambique, the Barcelona Center for International Health Research (CRESIB) and the

Fundacion Africa Viva, in Spain; and the Center for Vaccine Development at the University Of Maryland School Of Medicine, in the United States. The first section of work within this thesis is based on the Global Enteric Multicenter Study (GEMS). This project was a large and ambitious multicenter case-control study, aiming to assess the risk factors, microbiologic etiology and clinical presentation of MSD among children 0-59 months of age in Sub-Saharan Africa (Kenya, Mali, Mozambique, The Gambia) and SouthEast Asia (Bangladesh, India, and Pakistan). In the Manhiça site, 784 cases of MSD and 1545 matched controls were recruited. The study confirms rotavirus as the leading cause of diarrheal disease, and the massive underlying role that a vaccine against rotavirus could have in reducing diarrheal disease morbidity in Mozambique and other developing countries (first and second papers). Additional attention is also drawn to the importance of risk factors associated with moderate-to-severe diarrhea (third paper).

The second section within this thesis is based on two community surveys about attitudes and health care utilization in case of diarrhea performed during the above described case-control study, through interviews conducted with primary caretakers of children aged 0-59 months using a standardized questionnaire. The fourth paper of this thesis is a result of these surveys on health-care utilization in case of diarrhea. The paper describes the level of health care utilization, some components of the functioning of these health systems and the constraints associated with their use. This paper also provides an opportunity to understand the population perceptions about their attitudes and knowledge regarding diarrhea severity, prevention and treatment and highlights the importance of promoting breastfeeding and increased liquid intake during a diarrheal episode, in addition to oral rehydration solution as an essential part of any community based training program to improve the prognosis of diarrheal disease.

The final section of this theses involves a retrospective analysis of data recorded through the health facility morbidity surveillance system of all malnutrition cases in children aged less than five years of age seen at Manhiça's District Hospital during a one decade-long period (2001 to 2010). Detailed description of the clinical presentation of malnutrition diseases and risk factors associated with a bad prognosis are scarce in the Mozambican literature, and were unavailable for rural areas. These data provide invaluable guidance to help identify children who are at the highest risk of death and tailor accordingly and most efficiently the limited available resources in areas where health systems are chronically fragile. Particular attention is drawn to the description of the high burden of severe malnutrition cases detected at the Manhiça District Hospital.

6. HYPOTHESES AND OBJECTIVES

6.1. Hypotheses

- 6.1.1 Diarrheal disease will be among the principal causes of hospital admission and hospital related deaths in Manhiça
- 6.1.2 The knowledge of the etiologies behind diarrheal episodes admitted to hospital will confirm that the indiscriminate use of antibiotics may be inadequate to treat this clinical syndrome
- 6.1.3 The high burden of viral associated diarrheal episodes, and in particular of those caused by rotavirus, may call for the introduction of the rotavirus vaccine in areas like Manhiça
- 6.1.4 Use of health facilities in relation to diarrheal episodes will be adequate but community knowledge of the basic practices needed to handle the disease can be improved
- 6.1.5 Malnutrition, HIV infection and diarrheal will certainly overlap, and strategies designed to tackle any of these 3 diseases may likely have a positive impact for the other two

6.2 Objectives

6.2.1 General objective

To improve our understanding of the epidemiology of diarrhea, particularly to estimate the population-based burden, risk factors, microbiologic etiology and adverse clinical consequences of moderate-to-severe diarrhea among children 0-59 months of age in a sub-Saharan Africa area, to guide public health policy and target appropriate interventions.

6.2.2. Specific objectives

6.2.2.1 To describe the temporal trends and behavior of diarrhea among children who attend the outpatient/inpatient clinic of a rural district hospital

6.2.2.2 To estimate the population-based incidence of moderate-to-severe diarrhea in an area where population denominators are known at a community level as well as at the hospital

6.2.2.3 To determine the risk factors, etiology and clinical presentation of moderate-to-severe diarrhea and its related mortality among children 0-59 months of age in Manhiça District

6.2.2.4 To assess the perceptions and attitudes of primary caretakers about the dangers of diarrhea in children, and in particular to understand their understanding of the different types of diarrhea, its associated severity which are the defining events of dehydration, and finally how best to prevent it

6.2.2.5 To determine the ability of hospitals under Demographic Surveillance area to capture at least 75% of cases of moderate-to-severe diarrhea in the first 7 days of diarrheal disease

- 6.2.2.6 To determine the prevalence and describe clinical features of malnutrition among children less than five years of age attending the District Hospital of Manhiça
- 6.2.2.7 To establish the degree of overlap of malnutrition and diarrheal diseases in an area of high HIV incidence, and the specific microorganisms related with diarrheal episodes in such populations

7. MATERIALS AND METHODS

7.1 Study site, population and demographic surveillance system

The *Centro de Investigação em Saúde de Manhiça* (CISM) is located in Manhiça district (Maputo province, Southern Mozambique). The full description of geographic and socio-demographic characteristics of the study community has been detailed elsewhere (106-108). The CISM runs a demographic surveillance system (DSS) in this district since 1996, involving intensive and regular monitoring of a population of about 92,000 inhabitants in an area of around 500km². About a fifth (19%) of the study area inhabitants are children <5 years of age and all these children have a card with a permanent identification number issued by the DSS (109). All children involved in the studies within this thesis belong to Manhiça District. However as part of the multicenter GEMS study, one of the analyses involved children recruited from seven different study sites (described below).

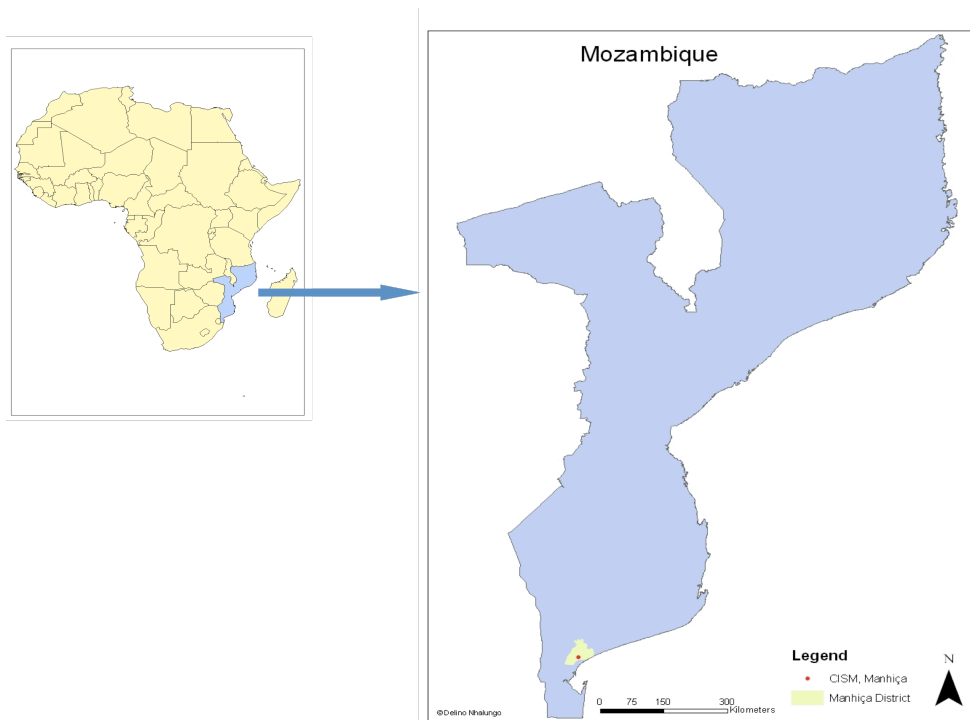


Figure 4: Map of Mozambique, and location of Manhiça District

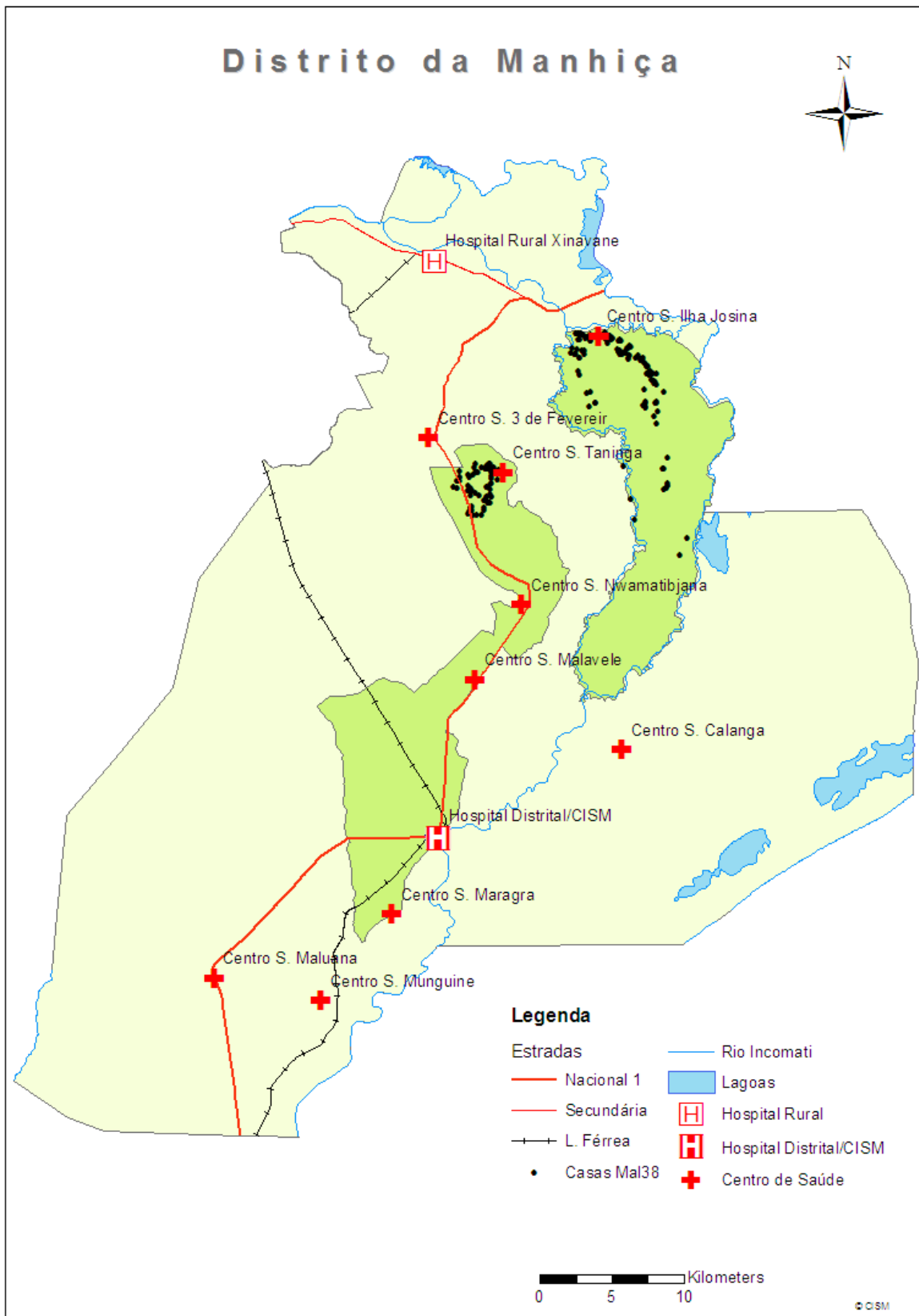


Figure 5: Map of Manhiça District

7.2 Healthcare facilities and morbidity surveillance system

The Manhiça District Hospital is adjacent to CISM and is one of the referral health centers in the area. It has an outpatient clinic, a mother and child health clinic, an emergency room, an HIV follow up program, a ward with 150 hospital beds, including a 16-bed specific malnutrition ward, and admits about between 3,000 to 4,500 children per year. There are five other nearby health posts (Maragra, Ilha Josina, Tanninga, Nwamatibzuana and Malavele) which deliver outpatient care and mother and child health services. All consultation for children less than five are free. A passive detection system has been progressively established since 1996 to cover all pediatric outpatient and inpatient visits to the above hospitals. Standardized forms are routinely completed for all outpatients and inpatients visits. Information collected include demographic, clinical (signs/symptoms and their duration), laboratory data, final diagnoses, antimalarial and antibiotic treatment received as well as the outcome. Malaria is screened in all febrile patients, and in patients with a history of fever in the preceding 24 hours prior to the arrival to hospital. When admission is required, a single blood culture is performed to all children under the age of two, and to older children with a temperature $\geq 39^{\circ}\text{C}$, with severe malnutrition or other signs of severe disease according to clinical judgment (110). By linking the information obtained through the morbidity surveillance platform to those provided by the demographic surveillance system (DSS), CISM is in a unique position to provide detailed descriptions of the health status of the community.

7.3 Laboratory facilities

Laboratory facilities at CISM include: parasitology, focused on malaria slide reading for detection of *plasmodium* spp; hematology and biochemistry, microbiology for general bacteriology and mycobacteriology, molecular biology and immunology. The panel

enteropathogens isolated in the case control study included bacteria [*Salmonella*, *Shigella*, *Campylobacter*, *Aeromonas*, *Vibrio spp.*, *Escherichia coli*, *Shigella*, pathotypes of diarrheagenic *E. coli* such as enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli*(EPEC), enteroaggregative *E. coli* (EAEC), protozoa agents (*Entamoeba histolytica*, *Giardia intestinalis*, and *Cryptosporidium*) and viral agents (rotavirus and enteric adenovirus, norovirus I/II, sapovirus and astrovirus)].



Figure 6: Triage of the Manhiça District Hospital, the referral health center in the DSS area

7.4 Methodology of papers

7.4.1 First, second and third papers

The first three papers within this thesis present results from a case-control study about the burden of diseases, risk factors, microbiologic etiology and clinical presentation of MSD among children aged 0-59 months between December 2007 and October 2011 in 7 countries, four in sub-Saharan Africa (Kenya, Mali, Mozambique, The Gambia) and further 3 in Southeast Asia (Bangladesh, India, Pakistan). The first paper of the study presents results of the data collected in the first three years of the study involving children recruited from the seven different study sites (multicenter analysis). The second paper describes the burden of diarrheal diseases and characterizes microbiologic etiology of moderate-to-severe diarrhea among the children living in Manhiça district (Mozambique site) during the whole period (2007-2011). The second paper also presents results of a retrospective analysis on the Minimum Community-based Incidence rates of acute diarrhea admitted to Manhiça's District Hospital between 2001 and 2012. The third paper describes the risk factors associated with the occurrence of moderate-to-severe diarrhea among the children living in Manhiça district during 2007-2011. Table 4 presents the periods involved in each of studies within this thesis.

7.4.2 Fourth paper

The fourth paper within this thesis is based on two community surveys about attitudes and health care utilization in case of diarrhea and moderate-to-severe diarrhea performed during the above described case-control study, through interviews conducted with primary caretakers of children aged 0-59 months using a standardized questionnaire. The first cross-sectional survey took place in Manhiça and surrounding villages between May 8th and June

28th, 2007. The second survey included a series of four repeated cross-sectional assessments that took place between February 16th, 2009 and December 30th, 2010.

Table 4: Chronology of activities and results presented in the papers within the thesis

Methodology		Paper	2001 to 2006	2007	2008	2009	2010	2011	2012
Diarrhea	MCBIRs retrospective analysis	2 nd							
	Case-control	Burden and etiology							
		Global burden and etiology (Multicentre analysis)	1 st						
		Risk factors	3 rd						
	HUAS	1 st Survey	4 th						
2 nd Survey									
Malnutrition	Retrospective analysis	5 th							

HUAS (Health utilization and attitude survey) MCBIRs (Community-based Incidence rates)

7.4.3 Fifth paper

The fifth paper presents a retrospective analysis of data collected through the morbidity surveillance system in outpatient and inpatient children aged less than five years of age. The study describes the prevalence of the different malnutrition syndromes seen as outpatients and the clinical features of severe malnutrition admitted to Manhiça District Hospital during the period 2001 to 2010.

7.5 Ethical issues

The studies presented in the first four papers of this thesis were approved by three different ethics committees before their initiation:

- Comitè d'Ètica i Investigacions Clíniques de l'Hospital Clínic de Barcelona; Barcelona, Spain
- Comité Nacional de Bioética para a Saúde (CNBS), Ministry of Health of Mozambique, Maputo, Mozambique
- Institutional Review Board for Human Subject Research at University of Maryland, Baltimore, United States

The study presented in the fifth paper of the thesis is a retrospective analyze of routinely collected clinical data in the context of normal clinical activity, and as such, did not have a specific ethical clearance.

7.6 Data management and statistical analysis

For the case-control and health care survey studies, the forms were scanned and stored as a pdf (Portable Document Format), after their initial transmission to the DCC (Data Coordinating Center) in Maryland (USA). When discrepancies were discovered during the verification process, a query was created and sent to the site supervisor who had to review, resolve and sign the form indicating that it was ready for a new submission to the DCC. Statistical analyses were performed using the Stata/SE software version 12.0.

For the fifth study, the questionnaires were double entered in FoxPro-designed databases (version 2.6, Microsoft Corporation, Redmond, WA, USA). Discrepancies in data entry were resolved by referring to the original forms. Statistical analyses were performed using STATA software (version 9.0, STATA Corporation, College Station, TX). The specific statistical methods utilized in every analysis are presented in detail in the full body of each of the articles.

8. ARTICLES

8.1 Article 1: Burden and etiology of diarrheal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study

Karen L Kotloff , James P Nataro, William C Blackwelder, Dilruba Nasrin, Tamer H Farag, Sandra Panchalingam, Yukun Wu, Samba O Sow, Dipika Sur, Robert F Breiman, Abu S G Faruque, Anita K M Zaidi, Debasish Saha, Pedro L Alonso, Boubou Tamboura, Doh anogo, Uma Onwuchekwa, Byomkesh Manna, Thandavarayan Ramamurthy, Suman Kanungo, John B Ochieng, Richard Omere, Joseph O Oundo, Anowar Hossain, Sumon K Das, Shahnawaz Ahmed, Shahida Qureshi, Farheen Quadri, Richard A Adegbola, Martin Antonio, M ahangir Hossain, Adebayo Akinsola, Inacio Mandomando, **Tacilta Nhampossa**, Sozinho Acácio, Kousick Biswas, Ciara E O'Reilly, Eric D Mintz, Lynette Y Berkeley, Khitam uhsen, Halvor Sommerfelt, Roy M Robins-Browne, Myron M Levine

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Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study

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Summary

Background Diarrhoeal diseases cause illness and death among children younger than 5 years in low-income countries. We designed the Global Enteric Multicenter Study (GEMS) to identify the aetiology and population-based burden of paediatric diarrhoeal disease in sub-Saharan Africa and south Asia.

Methods The GEMS is a 3-year, prospective, age-stratified, matched case-control study of moderate-to-severe diarrhoea in children aged 0–59 months residing in censused populations at four sites in Africa and three in Asia. We recruited children with moderate-to-severe diarrhoea seeking care at health centres along with one to three randomly selected matched community control children without diarrhoea. From patients with moderate-to-severe diarrhoea and controls, we obtained clinical and epidemiological data, anthropometric measurements, and a faecal sample to identify enteropathogens at enrolment; one follow-up home visit was made about 60 days later to ascertain vital status, clinical outcome, and interval growth.

Findings We enrolled 9439 children with moderate-to-severe diarrhoea and 13129 control children without diarrhoea. By analysing adjusted population attributable fractions, most attributable cases of moderate-to-severe diarrhoea were due to four pathogens: rotavirus, *Cryptosporidium*, enterotoxigenic *Escherichia coli* producing heat-stable toxin (ST-EPEC; with or without co-expression of heat-labile enterotoxin), and *Shigella*. Other pathogens were important in selected sites (eg, *Aeromonas*, *Vibrio cholerae* O1, *Campylobacter jejuni*). Odds of dying during follow-up were 8.5-fold higher in patients with moderate-to-severe diarrhoea than in controls (odds ratio 8.5, 95% CI 5.8–12.5, $p < 0.0001$); most deaths (167 [87.9%]) occurred during the first 2 years of life. Pathogens associated with increased risk of case death were ST-EPEC (hazard ratio [HR] 1.9; 0.99–3.5) and typical enteropathogenic *E coli* (HR 2.6; 1.6–4.1) in infants aged 0–11 months, and *Cryptosporidium* (HR 2.3; 1.3–4.3) in toddlers aged 12–23 months.

Interpretation Interventions targeting five pathogens (rotavirus, *Shigella*, ST-EPEC, *Cryptosporidium*, typical enteropathogenic *E coli*) can substantially reduce the burden of moderate-to-severe diarrhoea. New methods and accelerated implementation of existing interventions (rotavirus vaccine and zinc) are needed to prevent disease and improve outcomes.

Funding The Bill & Melinda Gates Foundation.

Introduction

Globally, one in ten child deaths result from diarrhoeal disease during the first 5 years of life, resulting in about 800 000 fatalities worldwide annually, most occurring in sub-Saharan Africa and south Asia.¹ Although diarrhoeal mortality remains unacceptably high, it is decreasing by about 4% per year,¹ whereas disease incidence is declining more modestly.² Interventions that target the main causes and focus on the most susceptible children should further accelerate these declines. To guide these efforts, robust data characterising the burden, risk factors, microbiological aetiology, sequelae, and case fatality of most life-threatening and disabling episodes

are essential; heretofore, such data have been scarce in regions with the highest child mortality. To address these knowledge gaps, we created the Global Enteric Multicenter Study (GEMS),³ the capstone component of which is a 3-year, prospective, age-stratified, matched case-control study of moderate-to-severe diarrhoea in children aged 0–59 months residing in censused populations and seeking care at medical facilities serving seven sites in sub-Saharan Africa and South Asia.⁴ We used a common research protocol with standardised epidemiological and microbiological methods to facilitate inter-site comparisons and allow aggregate estimates of aetiology and incidence.^{4–6}

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For the clinical protocol see
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 edu/GEMS/](http://medschool.umaryland.edu/GEMS/)

Methods

Study design and participants

The primary objective of GEMS was to measure the population-based burden, microbiological aetiology, and adverse clinical effects (eg, growth faltering and death) of moderate-to-severe diarrhoea in developing countries, overall and by age, pathogen, and site.⁴ The rationale and underlying epidemiological assumptions,^{3,7} and the clinical or epidemiological,⁴ microbiological,⁵ data management,⁸ and analytical⁶ methods have been detailed. Salient points are summarised below.

Seven field sites were selected among countries with moderate-to-high under-5 child mortality in Africa (Kenya, Mali, Mozambique, The Gambia) and Asia (Bangladesh, India, Pakistan).⁴ Each site provided a censused population, using a demographic surveillance system (DSS) in which fieldworkers visited each household to record births, deaths, and migrations two to four times every year, supplemented by weekly updates of births and deaths in children 0–59 months. GEMS targeted three age strata: infants (0–11 months), toddlers (12–23 months), and children (24–59 months); for each site and age stratum, we used the median population from DSS rounds during the case-control study for analyses. For case enrolment, sites selected sentinel hospitals or health centres (SHCs) where DSS children sought care for diarrhoeal illnesses.⁴

For 36 months between Dec 1, 2007, and March 3, 2011, all children aged 0–59 months belonging to the DSS population at every site who sought care at a SHC were screened for diarrhoea, defined as three or more loose stools within the previous 24 h.⁹ A GEMS clinician assessed each child with diarrhoea for eligibility. To be included, the episode had to be new (onset after ≥ 7 diarrhoea-free days), acute (onset within the previous 7 days), and fulfil at least one of the following criteria for moderate-to-severe diarrhoea: sunken eyes (confirmed by parent or caretaker as more than normal; loss of skin turgor (abdominal skin pinch with slow [≤ 2 s] or very slow [> 2 s] recoil); intravenous hydration administered or prescribed; dysentery (visible blood in loose stools); or admission to hospital with diarrhoea or dysentery.⁴ Although all cases meeting the definition of moderate-to-severe diarrhoea were documented, each site restricted enrolment to about the first nine eligible cases per age stratum per fortnight to maintain a manageable work flow throughout the study; we attempted to enrol 600 analysable case-control pairs per age stratum per site in 36 months, which would provide 80% power (two-sided test, 5% significance level) to find a significant difference for a site-stratum-specific comparison of the proportion of cases and controls in whom a specific enteropathogen is identified, if a pathogen is identified in 5.8% of cases and 2.5% of controls.^{4,6} For every enrolled case of moderate-to-severe diarrhoea, we aimed to enrol one to three control children without diarrhoea during a home visit, following an algorithm that increased the requisite controls according to the number of patients with moderate-to-severe diarrhoea enrolled in

that fortnight. Controls, matched to every individual patient with moderate-to-severe diarrhoea by age (± 2 months for patients aged 0–11 months and 12–23 months, and ± 4 months for patients aged 24–59 months), sex, and residence (same or nearby village or neighbourhood as the patient with diarrhoea), were randomly selected from the site's DSS database and enrolled within 14 days of the index case.⁴ Potential controls who had diarrhoea in the previous 7 days were ineligible.

The clinical protocol was approved by ethics committees at the University of Maryland, Baltimore, MD, USA, and at every field site. Written informed consent was obtained from the parent or primary caretaker of each participant before initiation of study activities.

Procedures

At enrolment, parents or primary caretakers of patients with moderate-to-severe diarrhoea and controls underwent standardised interviews to solicit demographic, epidemiological, and clinical information. GEMS staff, trained in standardised anthropometry, measured the child's length or height three times.⁴ Medical management at the SHC and clinical condition upon discharge were documented. Fieldworkers made one follow-up visit to every household of every patient with moderate-to-severe diarrhoea or control child about 60 days after enrolment (targeted range 50–90 days) to assess the child's vital status, capture interim medical events, and repeat anthropometric measurements.

At enrolment, each case and control provided at least 3 g of fresh stool, which within 1 h of passage was placed in cold storage until delivery to the laboratory. Additionally, if antibiotics were to be given to patients before stool was produced, we obtained two rectal swabs for bacterial culture pending passage of the whole stool for the remaining assays.⁴ Specimens were placed into transport media immediately (rectal swabs) or within 6 h of passage (whole stool aliquots) for bacterial culture and inoculated onto solid media within 18 h thereafter.⁴

Enteropathogens were identified using uniform methods.⁵ Bacterial agents (*Salmonella*, *Shigella*, *Campylobacter*, *Aeromonas*, and *Vibrio* spp) were detected using conventional culture techniques.⁵ Three putative *Escherichia coli* colonies from every stool were pooled and analysed by multiplex PCR that detect targets for enterotoxigenic (ETEC), enteroaggregative (EAEC), enteropathogenic (EPEC), and enterohaemorrhagic *E coli* (EHEC).⁵ The following gene targets defined 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), and EHEC (*eae* with *stx1*, *stx2*, or both, and without *bfpA*). Commercial immunoassays detected rotavirus (ELISA ProSpecT Rotavirus kit, Oxoid, Basingstoke, UK) and adenovirus (ProSpecT Adenovirus Microplate (Oxoid); adenovirus-

positive samples were tested for enteric adenovirus serotypes 40 and 41 (Premier Adenoclone kit, Meridian Bioscience, Cincinnati, OH, USA). Norovirus (genotypes I and II), sapovirus, and astrovirus were detected using multiplex reverse transcriptase (RT) PCR.⁵ Individual commercial immunoassays (TechLab, Inc, Blacksburg, VA, USA) detected *Giardia lamblia*, *Entamoeba histolytica* and *Cryptosporidium* spp.

Statistical analysis

We assessed associations of moderate-to-severe diarrhoea with potential pathogens using conditional logistic regression¹⁰ with a penalised likelihood approach,¹¹ taking into account the presence or absence of multiple pathogens as independent variables, we used odds ratios (ORs) and pathogen prevalence among patients with moderate-to-severe diarrhoea to calculate adjusted population attributable fractions (AFs)¹² to estimate pathogen-specific disease burden (expressed as number of cases and incidence rate). The adjusted AF is derived from a multiple conditional logistic regression model that includes other pathogens significantly associated with moderate-to-severe diarrhoea; thus it is the AF adjusted for presence of other pathogens.

Because samples from patients with moderate-to-severe diarrhoea were taken in roughly equal numbers during

each fortnight, irrespective of the number of cases of moderate-to-severe diarrhoea appearing at the SHCs, we estimated AF using weights defined as the number of eligible cases divided by the number of enrolled cases—ie, as the inverse of the sampling fraction for cases. We calculated weights separately for cases of moderate-to-severe diarrhoea with and without dysentery, to avoid any bias from overrepresentation or underrepresentation of cases with dysentery. We combined data for two or more adjacent fortnights to avoid having periods with either no patients with diarrhoea and dysentery enrolled or no patients with diarrhoea and without dysentery enrolled; typically, this resulted in the combination of data from several adjacent periods, so that weighting was based on numbers of cases in 16 weeks.

To estimate disease burden of moderate-to-severe diarrhoea in the population, we did brief surveys of health-care use serially during the case-control study in concert with each round of the DSS, using random samples of children.¹¹ We asked the parent or primary caretaker of children enrolled in every survey whether her or his child had a new episode of moderate-to-severe diarrhoea during the preceding 7 days, and, if so, the type of health care sought. After pooling data from serial surveys at each site, and applying sampling weights for surveyed children based on the number of children in

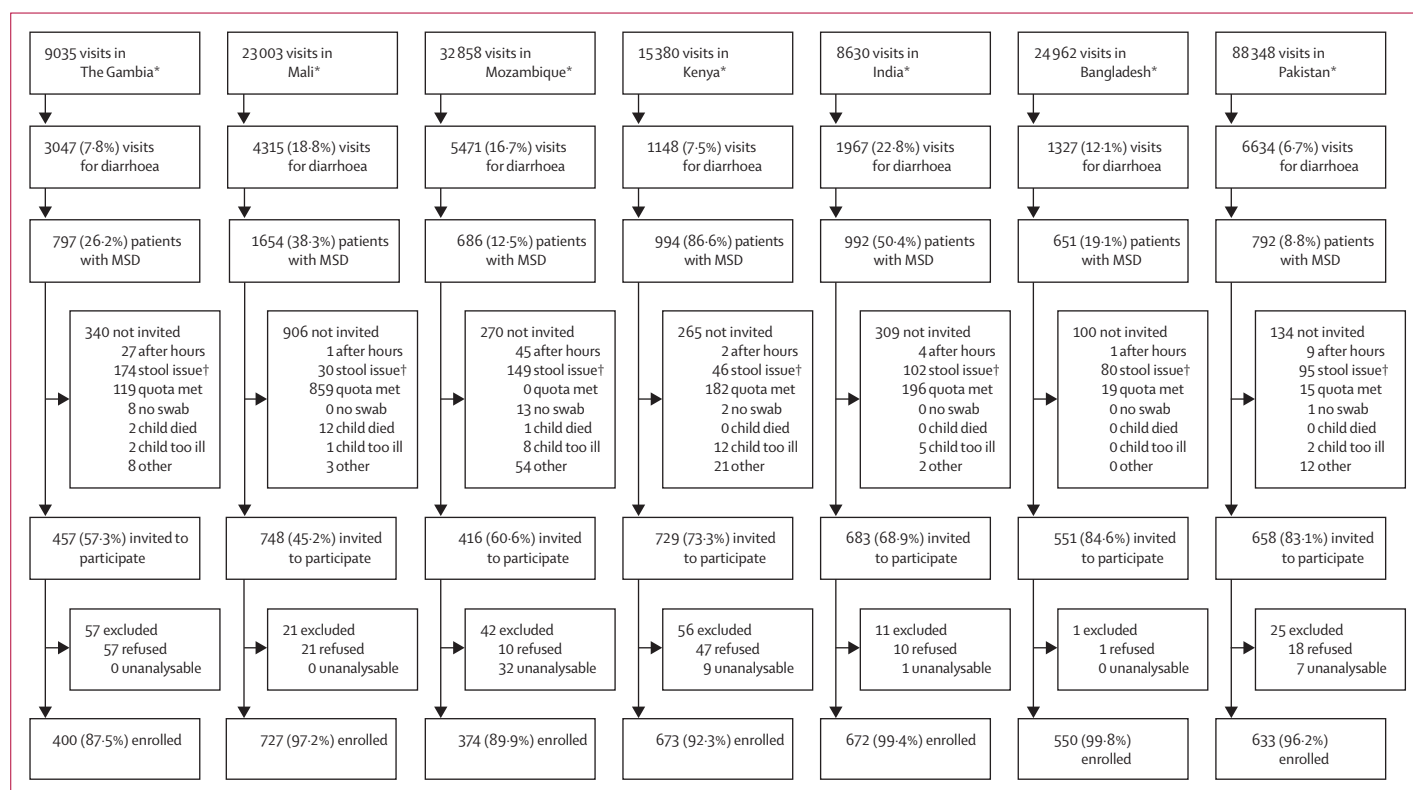


Figure 1: Study profile of children aged 0–11 months, by site

*Total visits to a sentinel health center by children in the demographic surveillance system area belonging to the corresponding age stratum. †Stool issues include no specimen, insufficient specimen, and improperly handled specimen.

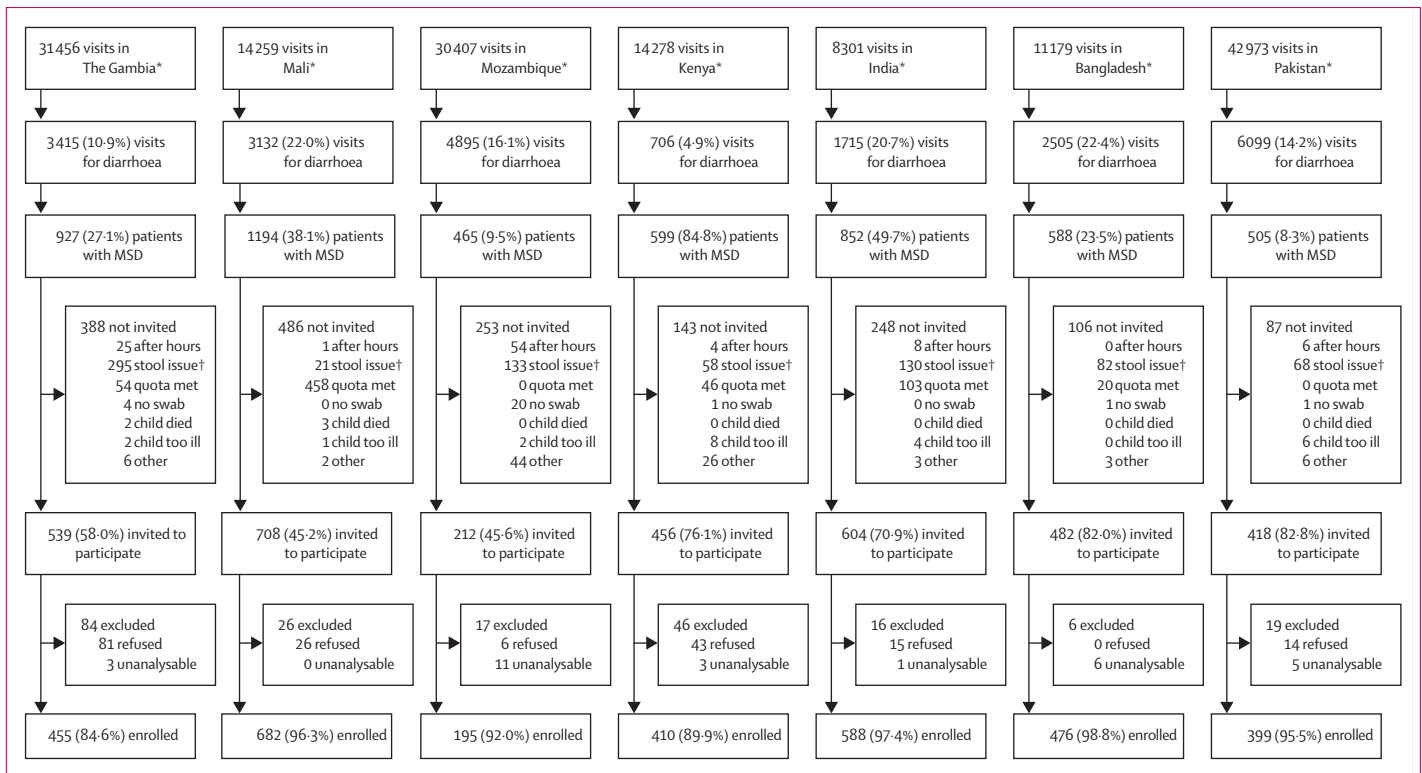


Figure 2: Study profile of children aged 12–23 months, by site

*Total visits to a sentinel health center by children in the demographic surveillance system area belonging to the corresponding age stratum. †Stool issues include no specimen, insufficient specimen, and improperly handled specimen.

each age–sex stratum in the DSS population, we calculated the proportion, designated “r”, of children with moderate-to-severe diarrhoea who were taken to an SHC at each site within 7 days of onset of diarrhoea.

For each site and age stratum, we estimated numbers of cases and incidence rates of moderate-to-severe diarrhoea per 100 child-years as follows. We calculated the annual number of cases of moderate-to-severe diarrhoea in the population as the number of eligible cases of moderate-to-severe diarrhoea recorded at SHCs during the 36-month study, divided by 3xr (with “r” defined as above). Division of this result by the median DSS population gave the moderate-to-severe diarrhoea incidence rate. To derive the number of cases and the incidence of moderate-to-severe diarrhoea attributable to a specific pathogen, the total cases and incidence rates of moderate-to-severe diarrhoea were multiplied by the pathogen’s weighted AF. Additionally, we calculated the incidence rates for all moderate-to-severe diarrhoea and for pathogen-specific attributable moderate-to-severe diarrhoea across sites by adding all cases of moderate-to-severe diarrhoea or all pathogen-specific attributable cases across sites and dividing by the sum of the sites’ populations.

We compared proportions of cases and controls who died during follow-up using conditional logistic regression or, when numbers of deaths were small (India and Bangladesh), Fisher’s exact test. We calculated

associations of pathogens with risk of dying in patients with moderate-to-severe diarrhoea, both unadjusted and adjusted for other pathogens and for site, using weighted proportional hazards (Cox) regression models to allow for different durations of follow-up, using the same weights as for analysis of AF.

The primary measure of growth in our analyses was the length or height-for-age Z (HAZ) score,¹³ which we derived using the median of three repeated measurements for every child at every visit according to WHO standards.¹⁴ We deleted implausible values for height and values that were inconsistent between enrolment and follow-up (appendix). Our analyses included only case-control sets with data on both enrolment and follow-up HAZ for the patients with moderate-to-severe diarrhoea. We calculated weighted means of enrolment HAZ score and change in HAZ score from enrolment to follow-up (δ HAZ) for patients with moderate-to-severe diarrhoea and controls, using the same type of weights as for AF analysis, redefined for the HAZ dataset. We compared enrolment HAZ scores for both controls and patients with moderate-to-severe diarrhoea using weighted paired *t* tests; we compared δ HAZ using weighted linear regression models for all possible matched pairs, adjusting for enrolment HAZ score and duration of follow-up, using jackknife estimates of standard error.

See Online for appendix

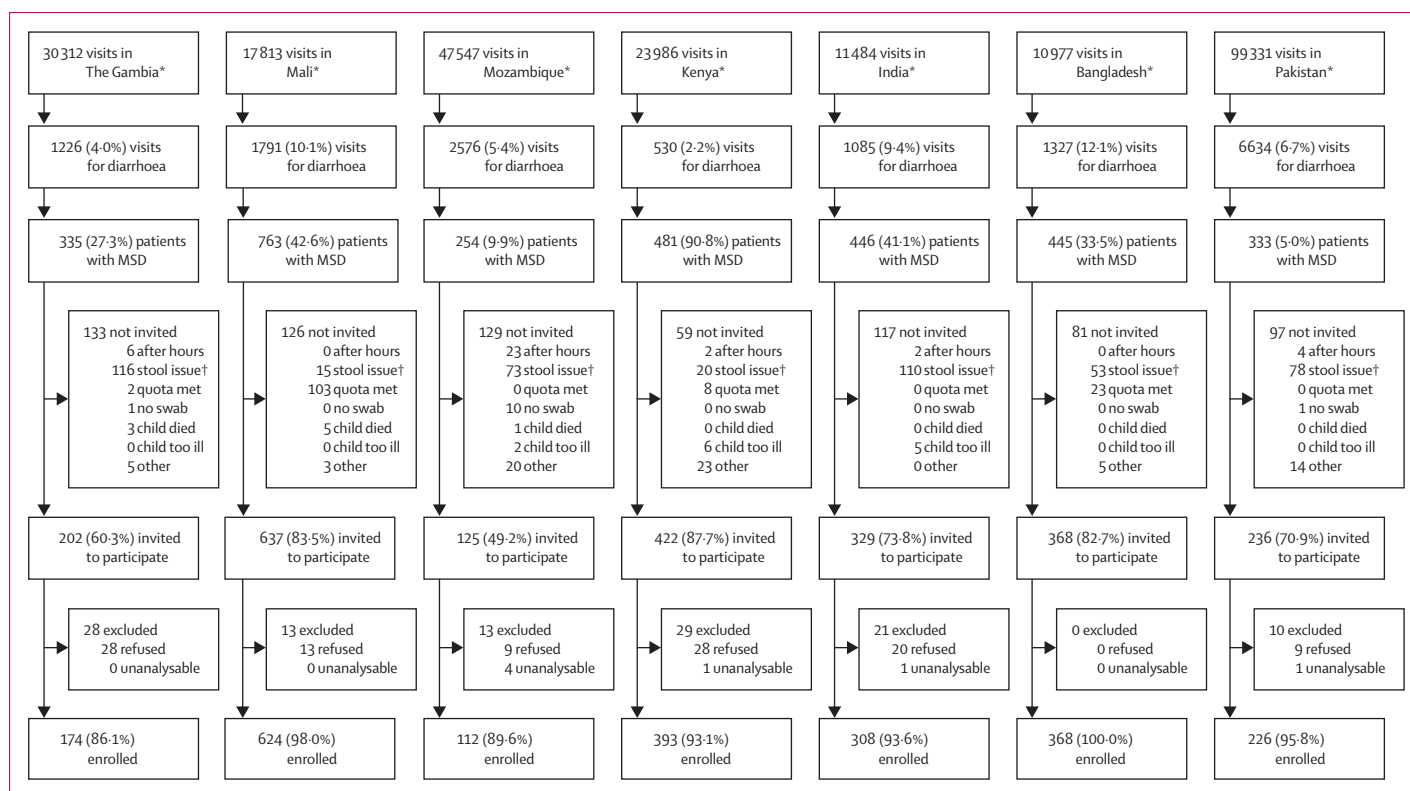


Figure 3: Study profile of children aged 24–59 months, by site

*Total visits to a sentinel health center by children in the demographic surveillance system area belonging to the corresponding age stratum. †Stool issues include no specimen, insufficient specimen, and improperly handled specimen.

We made no imputation of missing values. In regression models, we excluded observations with missing values from analysis. We deemed two-sided *p* values of 0.05 or lower to be significant.

Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

During the 36-month case-control study at seven DSS sites, children aged 0–59 months cumulatively contributed about 487 386 child-years of observation. They made 626 519 visits to an SHC, of which 66 009 (11%) were by children with diarrhoea (12.2% of the visits for infants, 14.7% of the visits for toddlers, and 6.3% of the visits for children); 14 753 children met enrolment criteria for moderate-to-severe diarrhoea (22% of diarrhoea cases), of whom 9980 were invited to participate (68% of eligible; figures 1–3). Of those invited, 9439 were included in this analysis (95%); 456 refused to participate (5%) and 85 did not have a matched control (1%) and were deemed unanalysable. Concomitantly, we enrolled 13 129 matched

controls. The household was visited about 60 days after enrolment for 8549 (91%) of patients with moderate-to-severe diarrhoea known to be alive at discharge from the SHC and 12 390 (94%) of enrolled control children. When we compared demographic and health indicators in cases and controls (table 1), controls in several sites and age groups belonged to a higher wealth quintile (eg, India and Pakistan), had greater access to improved water (Mali, Kenya, and The Gambia) and had more educated mothers (India) than did patients with moderate-to-severe diarrhoea.

Overall, we identified one or more putative pathogens in 7851 (83%) children with moderate-to-severe diarrhoea and in 9395 (72%) controls; two or more agents were identified in 4200 (45%) cases and 4075 (31%) controls. If one considers only the pathogens significantly associated with moderate-to-severe diarrhoea by conditional logistic regression and calculates the AF for the group of pathogens as a whole,⁶ the median proportion of episodes attributable to a pathogen was 44% (IQR 41–52) for infants, 47% (21–52) for toddlers, and 40% (23–53) for children. For some pathogens (eg, rotavirus, *Shigella*, *V cholerae* O1, adenovirus serotypes 40/41), nearly all infected children were symptomatic with moderate-to-severe diarrhoea, so a high percentage (about 90%) of cases of moderate-to-severe diarrhoea with the pathogen were attributable to that pathogen, compared with

	Basse, The Gambia		Bamako, Mali		Manhiça, Mozambique		Nyanza Province, Kenya		Kolkata, India		Mirzapur, Bangladesh		Karachi (Bin Qasim Town), Pakistan	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
0–11 months														
Number of patients	400	585	727	727	374	697	673	673	672	685	550	878	633	633
Mean age in months (SD)	7.8 (2.4)	7.9 (2.2)	7.3 (2.7)	7.0* (2.5)	6.5 (2.9)	6.7 (2.8)	6.5 (2.7)	6.3* (2.5)	6.8 (2.9)	6.8 (2.8)	7.2 (2.6)	7.0* (2.6)	6.1 (2.9)	6.0* (2.8)
Female individuals†	165 (41%)	255 (44%)	328 (45%)	328 (45%)	148 (40%)	272 (39%)	274 (41%)	274 (41%)	299 (45%)	307 (45%)	216 (39%)	346 (39%)	292 (46%)	292 (46%)
Caretaker completed primary school	25 (6%)	29 (5%)	118 (16%)	113 (16%)	84 (23%)	191 (28%)	361 (54%)	335 (50%)	404 (60%)	467 (68%)*	420 (76%)	659 (75%)	106 (17%)	121 (19%)
Mean wealth quintile ¹⁵	1.9	1.9	2.0	2.0	1.9	2.0	2.0	1.8	1.9	2.1*	2.1	2.0*	1.8	2.0*
Number-in household, median (range)	24 (3–129)	27* (3–120)	14 (2–71)	15 (3–110)	6 (2–42)	7* (2–73)	5 (2–17)	5 (2–40)	6 (2–18)	6 (3–20)	5 (2–23)	5 (2–30)	8 (2–50)	8 (2–66)
Access to improved water‡	164 (41%)	268 (46%)	471 (65%)	500 (69%)	108 (29%)	215 (31%)	68 (10%)	71 (11%)	328 (49%)	236 (34%)*	543 (99%)	869 (99%)	7 (1%)	4 (1%)
12–23 months														
Number of patients	455	639	682	695	195	391	410	621	588	598	476	761	399	676
Mean age in months (SD)	16.9 (3.4)	17.3* (3.3)	16.8 (3.5)	16.3* (3.2)	16.2 (3.2)	16.7* (3.1)	16.5 (3.5)	16.3 (3.1)	16.6 (3.4)	16.8 (3.1)	16.7 (3.3)	16.7 (3.1)	16.4 (3.3)	16.5 (2.9)
Female individuals†	208 (46%)	284 (44%)	300 (44%)	305 (44%)	85 (44%)	155 (40%)	186 (45%)	279 (45%)	259 (44%)	264 (44%)	209 (44%)	341 (45%)	166 (42%)	282 (42%)
Caretaker completed primary school	27 (6%)	33 (5%)	96 (14%)	103 (15%)	37 (19%)	87 (22%)	223 (54%)	320 (52%)	323 (55%)	379 (63%)*	356 (75%)	579 (76%)	58 (15%)	120 (18%)
Mean wealth quintile ¹⁵	2.0	2.1	2.1	2.0	1.9	1.9	1.9	1.9	1.8	2.0*	1.9	2.0	1.9	2.2*
Number in household, median (range)	23 (3–229)	30* (3–147)	13 (2–150)	13 (3–77)	6 (2–39)	6 (2–24)	5 (2–13)	6* (2–16)	6 (3–25)	5 (3–25)	5 (2–18)	5 (2–20)	8 (2–43)	8 (2–36)
Access to improved water	205 (45%)	289 (45%)	442 (65%)	492 (71%)*	57 (29%)	103 (27%)	33 (8%)	75 (12%)*	272 (46%)	208 (35%)*	472 (99%)	754 (99%)	2 (1%)	2 (0%)
24–59 months														
Number of patients	174	345	624	642	112	208	393	589	308	731	368	826	226	529
Mean age in months (SD)	31.8 (8.4)	31.6* (7.3)	35.6 (9.7)	35.1* (9.5)	33.8 (9.2)	33* (8.3)	36.1 (9.5)	35.8 (9.2)	35.6 (9.8)	35.6 (9.4)	34.9 (8.6)	34.9 (8.6)	35.0 (9.7)	35.4* (9.6)
Female individuals†	86 (49%)	174 (50%)	282 (45%)	290 (45%)	48 (43%)	74 (36%)	177 (45%)	266 (45%)	123 (40%)	299 (41%)	155 (42%)	355 (43%)	89 (39%)	209 (40%)
Caretaker completed primary school	16 (9%)	16 (5%)*	92 (15%)	92 (14%)	24 (22%)	45 (22%)	219 (56%)	320 (54%)	186 (60%)	458 (63%)	271 (74%)	608 (74%)	43 (19%)	103 (20%)
Mean wealth quintile ¹⁵	2.1	2.0	2.1	1.9*	2.0	2.2	2.1	2.1	1.8	2.1*	2.0	2.1	1.7	2.2*
Number-in household, median (range)	23 (3–100)	30* (3–180)	15 (2–100)	13* (2–81)	6 (2–16)	6* (3–72)	5 (2–14)	6* (2–25)	5 (3–15)	5 (2–18)	5 (2–18)	5* (2–22)	8 (3–26)	8 (2–60)
Access to improved water	62 (36%)	155 (45%)*	414 (66%)	446 (69%)	37 (33%)	58 (28%)	39 (10%)	60 (10%)	150 (49%)	248 (34%)*	366 (99%)	822 (100%)	0	0

SD=Standard deviation. *Significant difference, by conditional logistic regression ($p \leq 0.05$). †Statistical comparison not done because of exact sex matching; differences in overall proportions are due to numbers of multiple controls that differ by sex. ‡Improved water: the main source of drinking water for the household is either piped (into house or yard), public tap, tubewell (deep or shallow), covered well, protected spring, rainwater, or borehole, and is accessible within 15 min or less, roundtrip, and is available daily.

Table 1: Demographic features of cases with moderate-to-severe diarrhoea and their matched control

60–70% for agents such as *Cryptosporidium*, ST-EPEC, and *Aeromonas*.

Four pathogens were significantly associated with moderate-to-severe diarrhoea at all seven study sites in one or more age strata: rotavirus, *Cryptosporidium*, *Shigella*, and ST-EPEC (ST-only or LI/ST strains) (table 2). Most attributable episodes were associated with one of these pathogens. Rotavirus had the highest AF of any pathogen at every site during infancy. Although its AF generally diminished with age, rotavirus had the largest AF of any pathogen in toddlers at four sites, and at the

Mali and India sites even in the eldest stratum. *Cryptosporidium* had the second highest AF during infancy at five sites, persisting in importance, albeit at a lower level, during the second year of life at five sites; in the eldest stratum, *Cryptosporidium* was significantly associated with diarrhoea only in Kenya. By contrast, the adjusted AF of *Shigella* increased from infants to toddlers at every site, rising to the rank of first or second in AF at four sites in toddlers and five sites in the eldest stratum. In Mirzapur, Bangladesh, a GEMS site with rather high maternal education, low household crowding, and the

	Basse, The Gambia	Bamako, Mali	Manhiça, Mozambique	Nyanza Province, Kenya	Kolkata, India	Mirzapur, Bangladesh	Karachi (Bin Qasim Town), Pakistan
0–11 months							
Number of patients	400	727	374	673	672	550	633*
Rotavirus	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)
Cryptosporidium	11.7 (7.6–15.7)	14.0 (10.5–17.6)	14.7 (9.6–19.9)	9.0 (5.7–12.3)	11.8 (8.0–15.6)	5.3 (2.1–8.5)	5.6 (1.4–9.8)
ST-ETEC (ST-only or LT/ST)	4.9 (1.2–8.7)	3.6 (1.7–5.5)	..	7.0 (3.9–10.0)	3.0 (1.3–4.7)	1.4 (0.2–2.6)	7.0 (4.3–9.7)
Shigella	4.0 (1.7–6.4)	4.5 (2.4–6.6)	2.0 (0.7–3.3)	13.2 (10.3–16.1)	7.6 (5.3–9.9)
Norovirus GII	8.9 (4.3–13.4)
Aeromonas	9.7 (4.1–15.3)	11.3 (6.9–15.7)
Adenovirus 40/41	2.3 (0.6–4.0)	1.9 (0.8–3.0)	2.2 (0.4–4.0)	..	4.0 (2.3–5.6)	3.9 (2.0–5.7)	1.8 (0.8–2.9)
Campylobacter jejuni	9.0 (1.7–16.4)	6.7 (1.0–12.4)
Typical EPEC	5.2 (1.8–8.5)
Non-typhoidal Salmonella	4.2 (2.2–6.2)	..
Vibrio cholerae O1	3.1 (1.5–4.7)
Entamoeba histolytica	3.4 (0.4–6.4)	..
AF for all associated pathogens‡	51.9 (44.9–58.8)	39.5 (34.7–44.2)	41.6 (34.0–49.2)	41.1 (35.6–46.5)	43.7 (39.0–48.5)	58.4 (52.6–64.2)	52.3 (46.2–58.5)
12–23 months							
Number of patients	455	682	195	410	588	476	399
Rotavirus	17.0 (13.4–20.6)	11.8 (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)
Shigella	12.8 (8.7–16.9)	2.4 (0.8–4.0)	6.6 (2.5–10.7)†	4.6 (1.6–7.6)	7.2 (4.7–9.7)	52.2 (47.5–56.8)	12.5 (8.5–16.5)
Cryptosporidium	7.7 (4.5–10.9)	4.7 (1.0–8.3)	..	8.9 (5.4–12.4)	8.4 (3.6–13.2)	..	8.2 (4.3–12.2)
ST-ETEC (ST-only or LT/ST)	8.0 (3.1–13.0)	2.3 (0.2–4.4)	9.0 (3.6–14.3)	6.9 (3.6–10.3)	5.8 (3.1–8.5)	..	5.7 (2.4–8.9)
Aeromonas	11.9 (3.9–19.9)	9.5 (3.2–15.8)
Norovirus GII	8.7 (5.2–12.1)	4.7 (1.3–8.1)
Vibrio cholerae O1	3.4 (1.7–5.0)	1.4 (0.3–2.5)	7.5 (4.8–10.2)
Adenovirus 40/41	2.2 (0.7–3.7)	4.5 (2.7–6.3)	..	2.2 (0.2–4.1)
Non-typhoidal Salmonella	3.2 (0.5–6.0)
Typical EPEC	3.5 (0.3–6.7)
EAEC	9.9 (2.0–17.8)	..
AF for all associated pathogens‡	49.3 (43.0–55.6)	20.7 (15.8–25.7)	15.5 (8.9–22.2)	36.8 (30.2–43.4)	52.2 (46.5–57.8)	75.9 (70.7–81.2)	47.1 (40.2–54.1)
24–59 months							
Number of patients	174	624	112	393	308	368	226
Shigella	12.6 (6.4–18.8)	2.0 (0.0–19.5)	14.9 (7.9–21.9)	9.6 (6.1–13.1)	12.1 (7.8–16.4)	67.6 (61.3–73.8)	10.0 (4.6–15.4)
Aeromonas	18.3 (10.8–25.9)	24.1 (16.0–32.3)
Rotavirus	12.1 (6.6–17.6)	3.0 (0.0–21.9)	..	3.5 (1.3–5.6)	14.5 (10.2–18.7)
Vibrio cholerae O1	8.3 (2.8–13.9)	..	7.6 (4.6–10.6)	3.0 (1.0–5.0)	12.1 (7.7–16.5)
ST-ETEC (ST-only or LT/ST)	9.2 (3.1–15.3)	4.9 (2.0–7.9)	6.1 (3.1–9.1)	..	5.8 (1.2–10.3)
Campylobacter jejuni	9.9 (4.9–14.9)	..	16.1 (6.5–25.7)
Entamoeba histolytica	..	2.0 (0.0–18.5)
Norovirus GII	9.4 (2.6–16.2)
Non-typhoidal Salmonella	3.7 (1.2–6.1)
Cryptosporidium	2.5 (0.2–4.9)
Sapovirus	3.5 (1.2–5.8)
AF for all associated pathogens‡	39.9 (30.2–49.6)	6.8 (0.0–29.3)	23.3 (14.9–31.7)	23.3 (17.8–28.8)	46.9 (39.9–53.8)	75.6 (69.3–81.8)	52.6 (43.3–61.9)

ST=heat stable toxin. LT=heat labile toxin. ETEC=enterotoxigenic *Escherichia coli*. EPEC=enteropathogenic *E. coli*. AF=adjusted attributable fraction. MSD=moderate-to-severe diarrhoea. Pathogens included in the table are those that were significantly associated with MSD in weighted multiple conditional logistic regression analysis. *Astrovirus, though not included in table 2, was marginally significantly associated with MSD among infants in Pakistan (OR=1.8, p=0.0501). †Included in conditional logistic regression model even though not significant by jackknife because of a small number of controls (*Shigella* was isolated in 12/195 cases and 1/391 controls; p<0.0001 by Fisher's exact test). ‡The total attributable MSD for each stratum was determined from a model with all associated pathogens included;‡ it is not the sum of individual pathogen-specific AFs.

Table 2: Adjusted attributable fraction (AF, expressed as weighted percent of total episodes with 95% CI) of pathogens significantly associated with moderate-to-severe diarrhoea (MSD), by age stratum and site

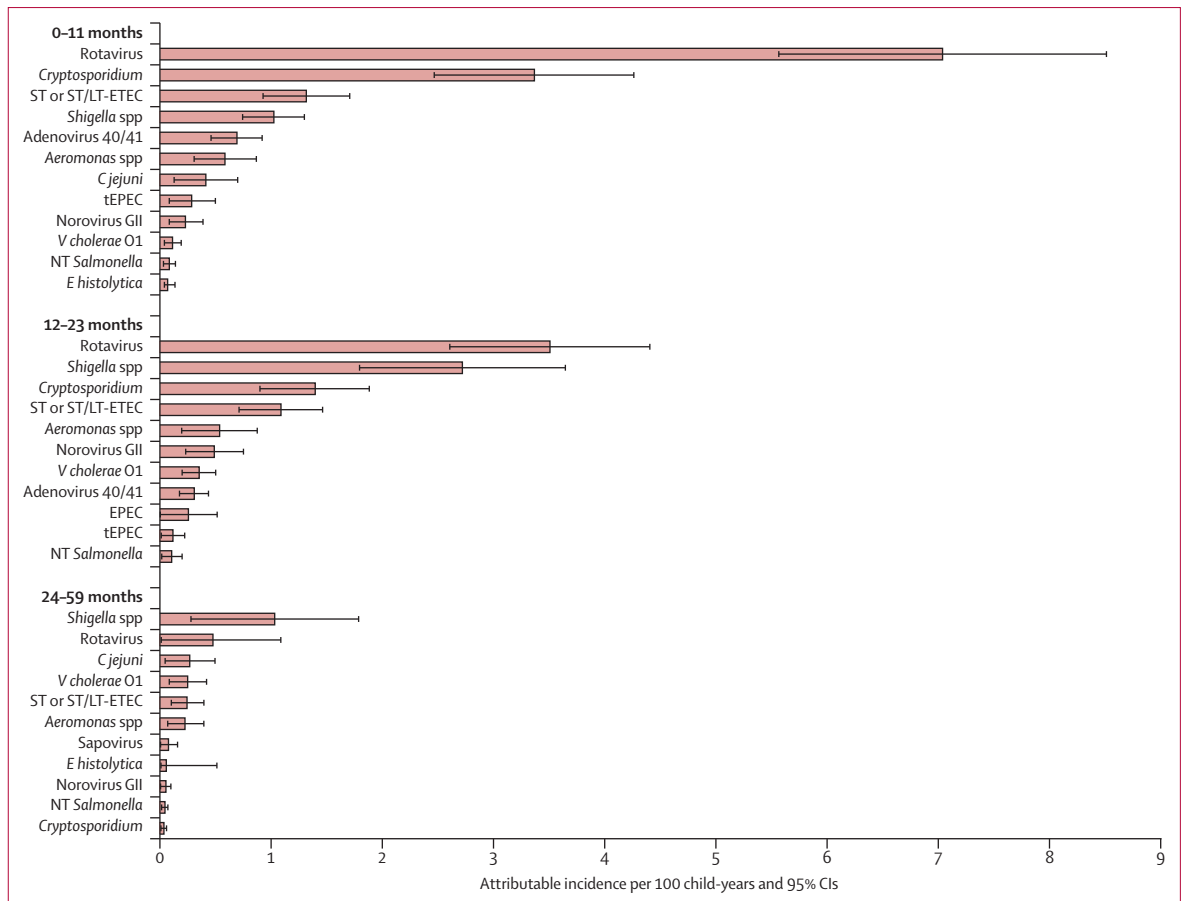


Figure 4: Attributable incidence of pathogen-specific moderate-to-severe diarrhoea per 100 child-years by age stratum, all sites combined
The bars show the incidence rates and the error bars show the 95% CIs.

highest reported access to improved water (table 1), *Shigella* was frequently isolated in patients with moderate-to-severe diarrhoea with dysentery (16.6% for infants, 66.0% for toddlers, and 78.4% for children) and in cases with watery diarrhoea (2.9% for infants, 20.6% for toddlers, and 43.4% for children). ST-EPEC was a significant pathogen at every site in at least one age stratum and in all age strata at four sites. By contrast, EPEC producing LT alone was not a significant cause of moderate-to-severe diarrhoea at any site or age stratum. A small proportion (<5%) of moderate-to-severe diarrhoea was attributable to adenovirus 40/41 at six sites during infancy, and in three sites during the second year of life.

Three enteropathogens showed regional importance. *Aeromonas* was a leading pathogen in the Pakistan and Bangladesh sites, with the peak AF at age 24–59 months. *V cholerae* O1 appeared in an age-escalating pattern in the three Asian sites plus Mozambique. *C jejuni* was significantly associated with moderate-to-severe diarrhoea in at least one age stratum at the three Asian sites.

Several pathogens had a smaller distribution, and were significantly associated with moderate-to-severe diarrhoea in two or fewer sites per age stratum, including norovirus

(GII genogroup), sapovirus, EAEC, typical EPEC, nontyphoidal *Salmonella*, and *E histolytica* (table 2). *Giardia* was not significantly positively associated with moderate-to-severe diarrhoea; to the contrary, in univariate analyses *Giardia* was identified significantly more frequently in controls than in patients with moderate-to-severe diarrhoea aged 12–59 months in ten of the 14 age-site strata.

When we estimated DSS-wide annual incidence rates of moderate-to-severe diarrhoea at all sites combined, rotavirus dominated during the first 2 years of life, with an incidence of moderate-to-severe diarrhoea during infancy (7.0 episodes per 100 child-years, 95% CI 5.4–8.5) that was more than double that of any other pathogen (figure 4). Generally, we noted two pathogens per age stratum whose incidence markedly exceeded the others: rotavirus and *Cryptosporidium* in infants; rotavirus and *Shigella* in toddlers; and *Shigella* and rotavirus in children (figure 4).

Regardless of the age stratum, the estimated incidence of moderate-to-severe diarrhoea was highest in India, next highest in Kenya and Mali, and lowest in The Gambia, Pakistan, Bangladesh, and Mozambique (table 3). The overall annual incidence of moderate-to-severe diarrhoea per 100 child-years was 30.8 (95% CI

24.8–36.8) for infants, 23.1 (95% CI 17.2–29.0) for toddlers, and 7.7 (95% CI 3.9–11.5) for children.

During follow-up within 90 days of enrolment, 190 (2.0%) deaths were detected in the 9439 children enrolled with moderate-to-severe diarrhoea, and 37 (0.3%) deaths were detected in the 13129 control children (OR 8.5, 95% CI 5.8–12.5, $p < 0.0001$; table 4). Mortality in children with moderate-to-severe diarrhoea was highest in the Mozambique site, followed by The Gambia and Kenya, Pakistan and Mali, and finally Bangladesh and India (table 4). Mortality in patients with moderate-to-severe diarrhoea exceeded mortality in controls at all sites and the differences were significant everywhere except in India (table 4). In patients with moderate-to-severe diarrhoea, 64 (34%) of deaths

occurred on days 0–7 after enrolment, 63 (33%) on days 8–21, and 63 (33%) after day 21; controls survived significantly longer than did patients with moderate-to-severe diarrhoea ($p < 0.0001$ by logrank test). Although 49 (26%) of deaths in patients with moderate-to-severe diarrhoea occurred during the enrolment encounter at the SHC and 36 (19%) during a subsequent medical contact, importantly, 105 (55%) occurred at home or outside of a medical facility.

Most deaths in patients with moderate-to-severe diarrhoea occurred in infants (107 [56%]) and toddlers (60 [32%]). Even so, the weighted risk of mortality remained high in the oldest stratum in The Gambia (1.8%), Kenya (2.3%), and Mozambique (3.9%). In multiple Cox regression analysis, pathogens associated

	Basse, The Gambia	Bamako, Mali	Manhiça, Mozambique	Nyanza Province, Kenya	Kolkata, India	Mirzapur, Bangladesh	Karachi (Bin Qasim Town), Pakistan
0–11 months							
MSD-total*	13.4 (7.8–19.1)	38.7 (17.0–60.5)	12.5 (6.2–18.8)	51.3 (29.0–73.6)	94.3 (56.4–132.2)	12.7 (6.5–18.9)	24.5 (12.2–36.8)
MSD-attributable	7.0 (3.9–10.0)	15.3 (6.5–24.1)	5.2 (2.4–8.0)	21.1 (11.5–30.6)	41.2 (24.1–58.4)	7.4 (3.7–11.1)	12.8 (6.2–19.4)
1	Rotavirus 3.2 (1.7–4.6)	Rotavirus 8.4 (3.5–13.3)	Rotavirus 3.5 (1.5–5.4)	Rotavirus 10.1 (5.4–14.8)	Rotavirus 25.4 (14.7–36.2)	Rotavirus 2.1 (1.0–3.2)	Rotavirus 5.5 (2.6–8.5)
2	Cryptosporidium 1.6 (0.7–2.4)	Cryptosporidium 5.4 (2.1–8.8)	Cryptosporidium 1.8 (0.7–3.0)	Cryptosporidium 4.6 (2.0–7.2)	Cryptosporidium 11.1 (5.4–16.9)	Shigella 1.7 (0.8–2.6)	Aeromonas 2.8 (1.0–4.5)
3	Norovirus GII 1.2 (0.4–2.0)	ST-EPEC 1.4 (0.3–2.5)	Adenovirus 40/41 0.3 (0.0–0.5)	Typical EPEC 2.7 (0.6–4.7)	Adenovirus 40/41 3.7 (1.6–5.9)	C jejuni 1.1 (0.1–2.2)	Shigella 1.9 (0.8–2.9)
4	ST-EPEC 0.7 (0.1–1.2)	Adenovirus 40/41 0.7 (0.1–1.3)	..	ST-EPEC 3.6 (1.4–5.8)	ST-EPEC 2.8 (0.9–4.8)	Cryptosporidium 0.7 (0.2–1.2)	ST-EPEC 1.7 (0.6–2.8)
5	Shigella 0.5 (0.2–0.9)	Shigella 2.3 (0.8–3.8)	Shigella 1.9 (0.4–3.3)	Adenovirus 40/41 0.5 (0.2–0.8)	C jejuni 1.7 (0.0–3.3)
6	Adenovirus 40/41 0.3 (0.1–0.6)	NT Salmonella 0.5 (0.2–0.9)	Cryptosporidium 1.4 (0.1–2.6)
7	E histolytica 0.5 (0.0–0.9)	Adenovirus 40/41 0.5 (0.1–0.8)
8	ST-EPEC 0.2 (0.0–0.4)	V cholerae O1 0.8 (0.2–1.3)
9	Aeromonas 1.2 (0.3–2.2)	..
12–23 months							
MSD-total*	19.2 (8.4–30.0)	34.3 (10.0–58.7)	7.8 (4.9–10.7)	22.1 (14.1–30.2)	48.8 (29.3–68.2)	16.2 (6.3–26.1)	16.6 (7.3–25.9)
MSD-attributable	9.5 (4.0–14.9)	7.1 (1.8–12.5)	1.2 (0.5–1.9)	8.2 (4.8–11.5)	25.4 (14.9–36.0)	12.3 (4.8–19.9)	7.8 (3.3–12.3)
1	Rotavirus 3.3 (1.3–5.2)	Rotavirus 4.1 (1.0–7.1)	ST-EPEC 0.7 (0.2–1.2)	Rotavirus 3.0 (1.6–4.3)	Rotavirus 12.4 (7.1–17.7)	Shigella 8.5 (3.3–13.7)	Shigella 2.1 (0.7–3.4)
2	Shigella 2.5 (0.9–4.1)	Cryptosporidium 1.6 (0.0–3.3)	Shigella 0.5 (0.1–0.9)	Cryptosporidium 2.0 (0.9–3.0)	Shigella 3.5 (1.7–5.4)	Rotavirus 3.0 (1.1–4.9)	Aeromonas 1.6 (0.2–2.9)
3	Norovirus GII 1.7 (0.5–2.8)	ST-EPEC 0.8 (0.0–1.7)	..	ST-EPEC 1.5 (0.6–2.5)	ST-EPEC 2.8 (1.1–4.6)	Aeromonas 1.9 (0.2–3.7)	Rotavirus 1.6 (0.6–2.7)
4	Cryptosporidium 1.5 (0.4–2.5)	Shigella 0.8 (0.0–1.6)	..	Shigella 1.0 (0.3–1.8)	Norovirus GII 2.3 (0.4–4.2)	V cholerae O1 0.2 (0.0–0.5)	Cryptosporidium 1.4 (0.4–2.4)
5	ST-EPEC 1.5 (0.3–2.8)	Typical EPEC 0.8 (0.0–1.5)	Adenovirus 40/41 2.2 (0.9–3.4)	EAEC 1.6 (0.0–3.2)	V cholerae O1 1.3 (0.4–2.1)
6	Adenovirus 40/41 0.4 (0.0–0.8)	NT Salmonella 0.7 (0.1–1.4)	V cholerae O1 1.6 (0.6–2.7)	..	ST-EPEC 0.9 (0.2–1.7)
7	Cryptosporidium 4.1 (1.2–6.9)	..	Adenovirus 40/41 O-4 (0.0–0.7)

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	Basse, The Gambia	Bamako, Mali	Manhiça, Mozambique	Nyanza Province, Kenya	Kolkata, India	Mirzapur, Bangladesh	Karachi (Bin Qasim Town), Pakistan
24–59 months							
MSD—total*	2.9 (1.0–4.9)	14.6 (0.0–31.3)	2.8 (0.1–5.6)	7.5 (5.3–9.6)	24.1 (0.6–47.6)	4.6 (0.0–9.3)	2.2 (0.8–3.6)
MSD—attributable	1.2 (0.3–2.0)	1.0 (0.0–4.5)	0.7 (0.0–1.3)	1.7 (1.1–2.4)	11.3 (0.1–22.4)	3.4 (0.0–7.1)	1.2 (0.4–1.9)
1	<i>Shigella</i> 0.4 (0.1–0.7)	Rotavirus 0.4 (0.0–3.2)	<i>Shigella</i> 0.4 (0.0–0.9)	<i>Shigella</i> 0.7 (0.4–1.1)	Rotavirus 3.5 (0.0–7.1)	<i>Shigella</i> 3.1 (0.0–6.3)	<i>Aeromonas</i> 0.5 (0.2–0.9)
2	Rotavirus 0.4 (0.1–0.6)	<i>Shigella</i> 0.3 (0.0–2.9)	<i>V cholerae</i> O1 0.2 (0.0–0.5)	ST-EPEC 0.4 (0.1–0.6)	<i>Shigella</i> 2.9 (0.0–5.9)	<i>Aeromonas</i> 0.8 (0.0–1.8)	<i>C jejuni</i> 0.4 (0.0–0.7)
3	Norovirus GII 0.3 (0.0–0.5)	<i>E histolytica</i> 0.3 (0.0–2.7)	..	Rotavirus 0.3 (0.1–0.4)	<i>C jejuni</i> 2.4 (0.0–5.0)	<i>V cholerae</i> O1 0.1 (0.0–0.3)	<i>V cholerae</i> O1 0.3 (0.1–0.5)
4	ST-EPEC 0.3 (0.0–0.5)	NT <i>Salmonella</i> 0.3 (0.1–0.5)	<i>V cholerae</i> O1 1.8 (0.0–3.8)	..	<i>Shigella</i> 0.2 (0.0–0.4)
5	<i>Cryptosporidium</i> 0.2 (0.0–0.4)	ST-EPEC 1.5 (0.0–3.1)	..	ST-EPEC 0.1 (0.0–0.3)
6	Sapovirus 0.8 (0.0–1.8)

MSD=moderate-to-severe diarrhoea; EPEC=enteropathogenic *Escherichia coli*. ST=heat stable toxin. ST-EPEC=either ST or ST/LT producing enterotoxigenic *E coli*. NT=non-typhoidal. AF=adjusted attributable fraction. Pathogens included in the table are those that were significantly associated with MSD in weighted multiple conditional logistic regression analysis. *The total attributable MSD for each stratum was identified for the group of pathogens together²³ and might be exceeded by the sum of each individual pathogen-specific AF, which does not account for co-infections.

Table 3: Weighted annual incidence (per 100 child-years) of total MSD, MSD attributable to a pathogen, and MSD attributable to a specific pathogen, with 95% confidence interval, by age stratum and site, in ordinal rank for specific pathogens

	Cases		Controls		OR (95% CI)
	Total*	Number of deaths (%)	Total*	Number of deaths (%)	
Manhiça, Mozambique	681	51 (7.5%)	1296	11 (0.85%)	13.4 (6.1–29.3)†
Basse, The Gambia	1029	39 (3.8%)	1569	7 (0.58%)	7.0 (3.0–16.5)†
Nyanza Province, Kenya	1476	52 (3.5%)	1883	11 (0.50%)	5.5 (2.8–10.7)†
Karachi (Bin Qasim Town), Pakistan	1258	16 (1.3%)	1838	1 (0.05%)	13.1 (0.99–172.4)†
Bamako, Mali	2033	23 (1.1%)	2064	5 (0.24%)	5.5 (1.8–16.5)†
Mirzapur, Bangladesh	1394	7 (0.50%)	2465	1 (0.04%)	12.4 (2.0–77.5)‡
Kolkata, India	1568	2 (0.13%)	2014	1 (0.05%)	2.6 (0.34–19.6)‡
All Sites Combined	9439	190 (2.0%)	13 129	37 (0.28%)	8.5 (5.8–12.5)†

OR=odds ratio. *Includes all enrolled patients with moderate-to-severe diarrhoea and their matched controls who met one of the following criteria: (1) the child was known to have died within 90 days of enrolment; (2) the child completed a follow-up visit between 50–90 days after enrolment; or (3) the child was located after day 90 and found to be alive. Note that the follow-up period in Kenya was actually 49–91 days. †ORs and 95% CIs from weighted conditional logistic regression; Mozambique (p<0.0001), The Gambia (p<0.0001), Kenya (p<0.0001), Mali (p=0.002), Pakistan (p=0.051), and p<0.0001 for all sites combined. ‡OR (unmatched) and 95% CI from a likelihood score method;¹⁶ p=0.004 for Bangladesh and p=0.58 for India, two-sided Fisher's exact test.

Table 4: Mortality in children with moderate-to-severe diarrhoea and their matched controls between enrolment and follow-up, by site

with a higher risk of dying in patients with moderate-to-severe diarrhoea were ST-EPEC and typical EPEC in infants and *Cryptosporidium* in toddlers (table 5). Adjusting for site, enrolment HAZ was inversely associated with risk of dying in patients with moderate-to-severe diarrhoea in all age groups, as follows: 0–11 months HR 0.62 (95% CI 0.54–0.72, p<0.0001); 12–23 months HR 0.74 (95% CI 0.63–0.87, p=0.0002);

and 24–59 months HR 0.47 (95% CI 0.38–0.57, p<0.0001).

Mean HAZ at enrolment in patients with moderate-to-severe diarrhoea and controls was considerably below the WHO reference for infants and, with one exception, deviated further from the reference at older ages (table 6). Linear growth faltering was especially marked at the Pakistan site. When we compared mean enrolment HAZ in cases of moderate-to-severe diarrhoea and their matched controls at the seven sites and three age strata, HAZ was significantly lower in cases than in controls in only two of 21 comparisons, both in infants (table 6). In pooled analysis, enrolment HAZ of cases and controls did not differ in the two older age strata but was significantly lower in infants with moderate-to-severe diarrhoea than in controls. Importantly, HAZ scores of moderate-to-severe diarrhoea cases decreased between enrolment and follow-up (ie, negative δ HAZ), with only one exception (Malian children aged 24–59 months); the decline was significantly greater in patients with moderate-to-severe diarrhoea than in controls in most site-age strata and in all age strata in the pooled analysis, after adjusting for enrolment HAZ and time to follow-up (table 6).

Discussion

Using a comprehensive panel of microbiological assays, GEMS was performed to better define the incidence, aetiology, and clinical outcome of moderate-to-severe paediatric diarrhoea in seven sites, located in regions where more than 80% of deaths in children younger than 5 years occur,¹⁷ and representing a range of health indicators (eg, malaria, HIV prevalence), health-care

	Pathogen present		Pathogen absent		Unadjusted†		Adjusted‡	
	Total	Number of deaths (%)	Total	Number of deaths (%)	HR (95% CI)	p value	HR (95% CI)	p value
0–11 months								
Typical EPEC	375	24 (6.4%)	3654	83 (2.3%)	2.8 (1.7–4.5)	<0.0001	2.6 (1.6–4.1)	0.0001
ST-EPEC	256	12 (4.7%)	3773	95 (2.5%)	2.0 (1.1–3.8)	0.03	1.9 (0.99–3.5)	0.05
<i>E histolytica</i>	115	5 (4.3%)	3914	102 (2.6%)	1.6 (0.64–4.2)	0.30
LT-EPEC	171	6 (3.5%)	3858	101 (2.6%)	1.2 (0.52–2.8)	0.67
Atypical EPEC	162	5 (3.1%)	3867	102 (2.6%)	1.0 (0.41–2.6)	0.93
<i>Cryptosporidium</i>	624	19 (3.0%)	3405	88 (2.6%)	1.2 (0.71–2.0)	0.52
EAEC	999	27 (2.7%)	3030	80 (2.6%)	0.92 (0.58–1.5)	0.71
<i>Giardia</i>	377	10 (2.7%)	3652	97 (2.7%)	1.1 (0.57–2.2)	0.74
Rotavirus	1016	25 (2.5%)	3013	82 (2.7%)	0.81 (0.51–1.3)	0.37
<i>Aeromonas</i>	251	5 (2.0%)	3778	102 (2.7%)	0.59 (0.24–1.5)	0.25
<i>C jejuni</i>	454	7 (1.5%)	3575	100 (2.8%)	0.55 (0.25–1.2)	0.13
12–23 months								
<i>E histolytica</i>	100	5 (5.0%)	3104	55 (1.8%)	3.5 (1.3–9.2)	0.01	2.1 (0.76–6.0)	0.15
<i>Cryptosporidium</i>	374	15 (4.0%)	2830	45 (1.6%)	2.6 (1.4–4.8)	0.002	2.3 (1.3–4.3)	0.006
EAEC	578	18 (3.1%)	2627	42 (1.6%)	1.7 (0.96–3.0)	0.07
Typical EPEC	232	7 (3.0%)	2973	53 (1.8%)	2.0 (0.87–4.5)	0.11
ST-EPEC	249	6 (2.4%)	2956	54 (1.8%)	1.2 (0.48–3.1)	0.68
<i>Shigella</i>	485	8 (1.6%)	2720	52 (1.9%)	0.89 (0.42–1.9)	0.77
<i>Giardia</i>	693	7 (1.0%)	2511	53 (2.1%)	0.51 (0.22–1.2)	0.12

HR=hazard ratio. ST=heat stable toxin. LT=heat labile toxin. ST-EPEC=either ST or ST/LT producing enterotoxigenic *E coli*. LT-EPEC=EPEC producing only LT. EPEC=enteropathogenic *E coli*. EAEC=enteroaggregative *E coli*. *Includes all enrolled patients with MSD and their matched controls who met one of the following criteria: (1) the child was known to have died within 90 days of enrolment. (2) the child completed a follow-up visit between 50–90 days after enrolment; or (3) the child was located after day 90 and found to be alive. Note that the follow-up period in Kenya was actually 49–91 days. †From Cox regression on data pooled from all sites, with presence or absence of pathogen as the only covariate; only pathogens that were isolated from at least five patients with MSD who died were included. ‡Adjusted for other pathogens, as well as study site; from Cox regression on data pooled from all sites, and including as covariates pathogens with $p < 0.05$ in unadjusted analysis and dichotomous variables for site.

Table 5: Weighted unadjusted and adjusted hazard ratios for selected pathogens and risk of death between enrolment and follow-up, in cases of moderate-to-severe diarrhoea (MSD)*

accessibility, economic development, and environmental conditions. By including matched control children without diarrhoea, we derived burden estimates adjusted for the occurrence of asymptomatic colonisation with enteropathogens often seen in children living in faecally contaminated environments,¹⁸ and derived an AF for every pathogen that was independently associated with moderate-to-severe diarrhoea in regression models, adjusting for interactions and confounding effects of co-infecting enteropathogens.⁶ Pathogen-specific adjusted AFs estimate the proportion of moderate-to-severe diarrhoea at our sites that could be prevented with targeted interventions such as effective vaccines.

Moderate-to-severe diarrhoea was common in the paediatric populations studied, producing more than 20 episodes per 100 child-years during each of the first 2 years of life. Three findings concerning children with moderate-to-severe diarrhoea are noteworthy. First, despite the wide array of putative pathogens that we detected, a small number contributed most attributable moderate-to-severe diarrhoea cases: rotavirus, *Cryptosporidium*, ST-EPEC, and *Shigella*, and, to a lesser extent, adenovirus

40/41. Several other pathogens were important only in Asia (*Aeromonas*) or Asia and Mozambique (*V cholerae* O1, *C jejuni*). Our findings support the notion that in Asia, *Aeromonas* causes diarrhoeal disease in young children.¹⁹ Second, children with moderate-to-severe diarrhoea experienced a substantial nutritional insult, evidenced by significantly more linear growth faltering during the follow-up period compared with their matched controls, even though, in 19 of the 21 site-strata, the mean enrolment HAZ scores of cases and controls were similar. Finally, compared with controls, moderate-to-severe diarrhoea cases had greatly (8.5 times) increased risk of dying during the follow-up period, and the risk was inversely associated with enrolment HAZ. Although risk of dying in patients with diarrhoea was greatest in GEMS sites with high HIV prevalence (Mozambique, Kenya), mortality in patients with moderate-to-severe diarrhoea was also substantial in our low HIV-prevalence rural sub-Saharan site (The Gambia), and moderate-to-severe diarrhoea was significantly associated with increased risk of death at all sites except India. Importantly, most deaths occurred outside health facilities and were detected only because the study

	0–11 months	12–23 months	24–59 months
Basse, The Gambia	293 cases; 423 controls	322 cases; 445 controls	127 cases; 237 controls
Enrolment HAZ			
Cases	-0.81 (-0.97 to -0.66)	-1.31 (-1.46 to -1.16)	-1.77 (-1.99 to -1.56)
Controls	-0.63 (-0.81 to -0.44)	-1.32 (-1.49 to -1.16)	-1.49 (-1.72 to -1.26)
ΔHAZ			
Cases	-0.31 (-0.38 to -0.23)	-0.29 (-0.34 to -0.23)	-0.07 (-0.14 to -0.01)
Controls	-0.34 (-0.42 to -0.26)	-0.14 (-0.19 to -0.08)	0.03 (-0.04 to 0.09)
Bamako, Mali	521 cases; 521 controls	520 cases; 528 controls	501 cases; 512 controls
Enrolment HAZ			
Cases	-0.60 (-0.72 to -0.48)*	-1.07 (-1.19 to -0.95)	-1.14 (-1.25 to -1.03)
Controls	-0.38 (-0.52 to -0.25)*	-1.04 (-1.15 to -0.92)	-1.08 (-1.18 to -0.97)
ΔHAZ			
Cases	-0.28 (-0.33 to -0.22)‡	-0.07 (-0.10 to -0.04)	0.07 (0.05 to 0.09)
Controls	-0.24 (-0.29 to -0.19)‡	-0.04 (-0.07 to -0.004)	0.09 (0.07 to 0.10)
Manhiça, Mozambique	230 cases; 405 controls	112 cases; 199 controls	59 cases; 104 controls
Enrolment HAZ			
Cases	-1.24 (-1.42 to -1.07)*	-1.65 (-1.90 to -1.39)	-1.55 (-1.93 to -1.18)
Controls	-1.03 (-1.17 to -0.90)*	-1.58 (-1.83 to -1.33)	-1.74 (-2.03 to -1.44)
ΔHAZ			
Cases	-0.21 (-0.31 to -0.11)‡	-0.41 (-0.50 to -0.31)	-0.15 (-0.24 to -0.06)‡
Controls	-0.12 (-0.20 to -0.05)‡	-0.07 (-0.15 to 0.01)	-0.003 (-0.06 to 0.05)‡
Nyanza Province, Kenya	560 cases; 560 controls	340 cases; 523 controls	339 cases; 519 controls
Enrolment HAZ			
Cases	-0.96 (-1.06 to -0.86)	-1.54 (-1.68 to -1.40)	-1.65 (-1.78 to -1.52)*
Controls	-0.93 (-1.03 to -0.83)	-1.64 (-1.78 to -1.50)	-1.76 (-1.87 to -1.66)*
ΔHAZ			
Cases	-0.35 (-0.40 to -0.30)	-0.30 (-0.34 to -0.26)	-0.13 (-0.16 to -0.11)
Controls	-0.21 (-0.25 to -0.17)	-0.10 (-0.14 to -0.06)	-0.008 (-0.03 to 0.01)
Kolkata, India	623 cases; 635 controls	542 cases; 551 controls	295 cases; 690 controls
Enrolment HAZ			
Cases	-1.02 (-1.10 to -0.93)	-1.49 (-1.60 to -1.39)	-1.68 (-1.83 to -1.54)
Controls	-1.02 (-1.11 to -0.93)	-1.41 (-1.51 to -1.31)	-1.70 (-1.81 to -1.59)
ΔHAZ			
Cases	-0.29 (-0.32 to -0.25)‡	-0.14 (-0.17 to -0.11)	-0.05 (-0.08 to -0.02)
Controls	-0.34 (-0.38 to -0.29)‡	-0.11 (-0.14 to -0.09)	-0.02 (-0.04 to 0.005)
Mirzapur, Bangladesh	523 cases; 824 controls	455 cases; 712 controls	352 cases; 780 controls
Enrolment HAZ			
Cases	-1.06 (-1.16 to -0.96)	-1.32 (-1.43 to -1.22)	-1.50 (-1.61 to -1.39)
Controls	-1.07 (-1.15 to -0.99)	-1.36 (-1.45 to -1.28)	-1.53 (-1.63 to -1.43)
ΔHAZ			
Cases	-0.28 (-0.32 to -0.23)	-0.18 (-0.21 to -0.14)	-0.15 (-0.18 to -0.12)
Controls	-0.16 (-0.20 to -0.12)	-0.06 (-0.09 to -0.03)	-0.02 (-0.03 to 0.0001)
Karachi (Bin Qasim Town), Pakistan	397 cases; 397 controls	291 cases; 453 controls	170 cases; 353 controls
Enrolment HAZ			
Cases	-1.61 (-1.74 to -1.48)	-2.16 (-2.34 to -1.99)	-2.43 (-2.64 to -2.22)
Controls	-1.50 (-1.63 to -1.37)	-2.06 (-2.21 to -1.92)	-2.28 (-2.44 to -2.13)

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included a follow-up home visit about 60 days after enrolment.³⁷ Although we did not undertake surveillance to identify interim events affecting the child's outcome, our findings suggest that moderate-to-severe diarrhoea might contribute to the causal pathway to death, probably influenced by its associated nutritional derangement.

Evidence from GEMS that rotavirus is the most common cause of moderate-to-severe diarrhoea at every site during the first year of life renders optimism that the decision by the GAVI Alliance to make rotavirus vaccines available to the world's poorest countries²⁰ will substantially benefit global child health. Surveillance at GEMS sites could ascertain the effect of programmatic use of rotavirus vaccine including whether vaccine-derived protection persists beyond infancy. Although the point estimate of rotavirus vaccine efficacy recorded in field trials is distinctly lower in resource-poor countries than in resource-rich settings,²¹ high levels of rotavirus vaccine coverage might nevertheless achieve profound effects.²²

Remarkably, *Cryptosporidium* was a significant pathogen at all sites regardless of HIV prevalence, and the second most common pathogen in infants. Although often reported as a cause of life-threatening illness in individuals with HIV/AIDS and of diarrhoea and malnutrition in young children in sub-Saharan Africa,²³ the disease burden in the general paediatric population has been poorly quantified (particularly in Asia). The importance of *Cryptosporidium* in GEMS, and its association with death during the ensuing 2–3 months in toddlers aged 12–23 months, is consistent with previous findings of a cohort study in Guinea-Bissau,²⁴ a low HIV-prevalent area. These findings highlight the need to develop resources to diagnose, treat, and prevent cryptosporidiosis in resource-poor settings.

EPEC has been associated with acute and persistent diarrhoea leading to nutritional faltering and death.²⁵ However, the frequent detection in children without diarrhoea and high prevalence of breastfeeding (known to be protective) in many study populations are among the factors that have obfuscated estimates of EPEC disease burden in developing countries.²⁶ The ability of typical EPEC to cause diarrhoea is well established;²⁷ the virulence of atypical EPEC is less certain.²⁸ In GEMS we noted no association between atypical EPEC and moderate-to-severe diarrhoea, whereas typical EPEC was significantly associated with moderate-to-severe diarrhoea during the first 2 years of life at one site (Kenya). When we limited our analysis to cases, typical EPEC was significantly associated with death in infants aged 0–11 months. Efforts to prevent diarrhoea-associated morbidity and mortality might have to include pathogens such as typical EPEC that are not strongly associated with moderate-to-severe diarrhoea overall, but, when present in patients with moderate-to-severe diarrhoea, seem to be disproportionately associated with poor outcomes. Since linear growth faltering was a risk factor

for death in GEMS, nutritional rehabilitation should be part of case management algorithms for diarrhoea.

Limitations of the GEMS design have been described,⁴ and others are noted here. For one, antibiotic use before the SHC visit could have diminished the yield of bacterial cultures. Conversely, pathogens identified with molecular tests might be overdiagnosed in cases and controls.²⁹ The GEMS sites are endemic for many enteropathogens, creating an environment in which the same enteropathogens are commonly detected in controls without diarrhoea, which underestimates AF.^{18,30} That children with moderate-to-severe diarrhoea are at high risk for linear growth faltering and death is of utmost importance and demands further investigation. Nonetheless, one must be circumspect in assuming causality, particularly because our study did not define the contribution of intercurrent illnesses, nor did it factor in comorbidities (eg, HIV, malnutrition) that might have contributed both to the episode of moderate-to-severe diarrhoea and the adverse outcome. Our matched-pair analysis of the effect of an episode of moderate-to-severe diarrhoea on linear growth required exclusion of about 20% of individuals (appendix), which could theoretically introduce bias. Therefore, it was reassuring that baseline HAZ scores did not differ significantly between included and excluded children, nor did socio-demographic variables (appendix).

Finally, since the use of SHCs by children in the DSS population was low, our estimates of overall and pathogen-specific moderate-to-severe diarrhoea incidence might be imprecise. However, except for India, the site-specific incidence rates of rotavirus moderate-to-severe diarrhoea for infants (1.9 to 10.0 episodes per 100 child-years) were similar to published estimates of moderate-to-severe rotavirus diarrhoea (albeit derived using various definitions) from developing countries in Africa and south Asia.^{31,32} The India site had the lowest SHC use for moderate-to-severe diarrhoea (r value), possibly due to some unknown source of bias, and a lower r increases the variability of the point estimate. Second, in the crowded slums of Kolkata, where there are open sewage drains and the highest population density of any GEMS site (18 601 individuals per km²),³³ spread of enteric pathogens such as rotavirus and *Shigella* might be enhanced through several methods of transmission.³⁴ Finally, despite extensive training and standardisation of clinical criteria for moderate-to-severe diarrhoea, some site-to-site variation occurred. In India, 50% of DSS children with diarrhoea seeking care at the SHC were deemed to have moderate-to-severe diarrhoea, more often than any other site except Kenya. These factors might have contributed to higher estimates of both moderate-to-severe diarrhoea incidence and the proportion of moderate-to-severe diarrhoea attributable to specific pathogens in India than at other GEMS sites.

Our results documenting the substantial burden of moderate-to-severe diarrhoea in sub-Saharan Africa and south Asia and its close association to malnutrition show

	0-11 months	12-23 months	24-59 months
(Continued from previous page)			
δ HAZ			
Cases	-0.26 (-0.32 to -0.20)	-0.19 (-0.23 to -0.14)	-0.05 (-0.09 to -0.006)
Controls	-0.25 (-0.31 to -0.19)	-0.15 (-0.19 to -0.11)	0.03 (-0.001 to 0.05)
All sites combined	3147 cases; 3765 controls	2582 cases; 3411 controls	1843 cases; 3195 controls
Enrolment HAZ			
Cases	-0.98 (-1.03 to -0.93)†	-1.43 (-1.48 to -1.37)	-1.60 (-1.66 to -1.53)
Controls	-0.86 (-0.91 to -0.81)†	-1.41 (-1.47 to -1.35)	-1.57 (-1.63 to -1.51)
δ HAZ			
Cases	-0.29 (-0.31 to -0.27)	-0.19 (-0.21 to -0.18)	-0.06 (-0.07 to -0.04)
Controls	-0.25 (-0.27 to -0.23)	-0.09 (-0.11 to -0.08)	0.02 (0.009 to 0.03)

Data are weighted mean (95% CI). δ HAZ denotes HAZ at the follow-up visit minus HAZ at enrolment. *The follow-up period in Kenya was 49-91 days. Enrolment HAZ in cases vs controls was compared by weighted paired t test: †p=0.01-0.04 and ‡p=0.0009; all other p values >0.05. Δ HAZ in cases vs controls was compared by weighted linear regression, adjusting for enrolment HAZ and duration to follow-up: †p=0.01 to <0.05; ‡p=0.0001-0.008; ||p<0.0001, all other p values >0.05.

Table 6: Comparison of enrolment length or height for age Z score (HAZ), and change in HAZ (Δ HAZ) between enrolment and follow-up 50-90 days later,* between cases with moderate-to-severe diarrhoea and their matched controls, by site

Panel: Research in context

Systematic review

We searched PubMed for English, French, Spanish, and Portuguese publications using various combinations of the terms "diarrhea," "gastroenteritis," "diarrheal disease," "pediatric," "etiology," "microbiology," "growth faltering," and "malnutrition". We focused mainly on studies published since 1980 but included older reports where relevant. To identify additional publications we perused the reference lists of the original and review articles. Epidemiological studies were critically reviewed to detect methodological limitations and microbiological techniques were scrutinised. We also made judgments about the interest and relevance of studies for the well informed general clinician and public health practitioner.

From 1980 through roughly 2004, various case-control and small cohort studies investigated the aetiology of paediatric diarrhoea in low-income countries. Many studies had methodological inadequacies and arrived at disparate conclusions, making it difficult to prioritise the relative importance of different pathogens. Thus, there was no consensus on what specific diarrhoeal disease pathogens should be targeted for prevention. By contrast, agreement existed on the need for a well designed study to obtain information on the aetiology and burden of more severe forms of diarrhoeal disease to guide global investment and implementation decisions.

Interpretation

The Global Enteric Multicenter Study (GEMS) was designed to overcome drawbacks of earlier studies and determine the aetiology and population-based burden of paediatric diarrhoeal disease. Our findings demonstrate that interventions targeting only five pathogens can substantially reduce the burden of moderate-to-severe diarrhoea. GEMS data will guide investment and help prioritise strategies to mitigate the morbidity and mortality of paediatric diarrhoeal disease.

that preventive strategies targeting as few as four pathogens could potentially reduce this disease and its sequelae by about 40% during the first 2 years of life. Accordingly, an urgent need exists to accelerate introduction of existing interventions with proven effectiveness, such as rotavirus

vaccination and adjunct treatment of acute diarrhoea with zinc, to develop additional strategies with potential high impact, such as new vaccines, and to revitalise diarrhoeal disease case management algorithms shown to reduce malnutrition (panel).^{35,36}

Contributors

MML conceived the project and acquired the grant funds. MML, KLK, and JPN designed the protocol. WCB did the statistical analysis with YW, DN, HS, THF, and KM. KLK, JPN, DN, THF, SP, LB, SOS, DSu, RFB, ASGF, AKMZ, RAA, DSah, PLA, EDM, and CEOR planned and supervised the study. DSan, SK, RO, SKD, SA, FQ, AA, TN, and SA coordinated clinical data collection; BT, TR, JBO, JOO, AH, SQ, MA, IM, and RMR-B did the laboratory assays; and UO, BM, MJH, and KB participated in data management. KLK, WCB, DN, THF, YW, KM, JPN, and MML had full access to all the data in the study and did data analysis; KLK wrote the report with input from all authors and had final responsibility for the decision to submit for publication. All authors reviewed the draft and approved the decision to submit for publication.

Conflicts of interest

We declare that we have no conflicts of interest.

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**8.2 Article 2: Diarrheal disease in rural Mozambique. Part I: Burden and etiology of
diarrheal disease among children aged 0-59 months**

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Abstract

Background

Diarrheal disease remains a leading cause of illness and death, particularly in low-income countries. However, detailed aetiology status and accurate surveillance systems that allow prioritisation of tools and monitoring impact have been remarkably scarce

Methods

Trends of diarrhea-related burden of disease were estimated in children aged 0-59 months during the period 2001-2012. Yearly minimum community-based incidence rates of admitted acute diarrhea recorded through the health facility morbidity surveillance system was related to a demography surveillance system. A prospective, age-stratified and matched case-control study was conducted during 2007-2011. Clinical, epidemiology, anthropometric measurement and faecal samples obtained from recruited children were used to estimate moderate-to-severe diarrhea (MSD) attributable fractions.

Results

Over the last decade the incidence of acute diarrhea has dropped by about 80%. Incidence of MSD per 100 child years at risk for the period 2007-2011 was 9.85, 7.73 and 2.10 for children aged 0-11, 12-23 and 24-59 months respectively. By analyzing adjusted population attributable fractions, most cases of MSD were due to rotavirus, *Cryptosporidium*, ETEC ST (ST only or ST/LT), *Shigella* and Adenovirus 40/41.

Conclusions

Interventions targeting the principal etiologies causing MSD can reduce the burden of disease. Accelerating the introduction of rotavirus vaccine is a priority.

Introduction

Diarrheal disease remains a major contributor to illness and death among children less than five years in developing countries. Indeed, pediatric diarrheal disease still accounts for over 800.000 annual deaths globally, *circa* 11% of the 7.6 million estimated annual global child deaths (1). However, a review of studies from the past two decades suggests that mortality from diarrhea has been steadily decreasing worldwide, mainly due to the implementation of effective control programs and an improved socioeconomic situation (1-4).

Diarrhea may be caused by infectious organisms, including viruses, bacteria, protozoa, and helminths (5). The etiological agents of diarrhea vary greatly depending on country, region and community, so their knowledge is essential to inform prevention and control programs. Information from Mozambique is relatively scarce, but confirms that diarrheal diseases are a significant contributor to morbidity and mortality, especially among younger children (6-9). In addition, to our knowledge no study has specifically investigated moderate-to-severe diarrhoea (MSD) episodes. The aim of the present study was to describe the burden and incidence of diarrheal disease and identify the pathogens causing MSD among children aged <5 years in a rural Mozambican area, as part of the Global Enteric Multicenter Study (GEMS) (10).

Methods

Study area and population

The study was conducted, in the District of Manhiça, a rural area located 80 kilometers north of the capital of Mozambique, Maputo. The climate is subtropical with two distinct seasons: a warm and rainy season from November to April and a generally cooler and drier season during the rest of the year. The average annual temperature ranges from 22°C to 24°C and annual rainfall ranges from 600 to 1000mm. Community prevalence of HIV/AIDS in Manhiça is amongst the highest in the world, with prevalence rates in women in child-bearing age as high as 40% in the district (11). Diarrhea is the third leading cause of hospital admission among children aged 0-14 years and the fourth leading cause of death among children between 12 and 59 months (6), according to verbal autopsies performed in the area. The Manhiça district has about 150,000 inhabitants, and the *Centro de Investigação em Saúde da Manhica* (CISM) runs a demographic surveillance system (DSS) in this district since 1996, involving intensive and regular monitoring of a population of about 92,000 inhabitants in an area of around 500km². About a fifth (19%) of the study area inhabitants are children aged <5 years (12). A round-the-clock morbidity surveillance system, covering both pediatric outpatient and hospital admission was established in 1996 at the Manhiça District Hospital-MDH (the main facility and the only one with admission facilities) and has progressively integrated five other rural health post (13). Clinical data for all children under 15 years of age are routinely collected by a trained medical officer or physician using standardized forms.

Study design

We estimated the incidence of acute diarrhea episodes in children aged 0-59 months admitted to MDH between the years 2001 and 2012 based on an ongoing health facility morbidity surveillance system related to the DSS. A case-control study was conducted between

December 2007, and October 2011. All children aged 0–59 months belonging to the DSS population who sought care at the health facilities within DSS area were screened for diarrhoea, defined as three or more loose stools within the previous 24h. Study clinician assessed each child with diarrhoea for eligibility. To be included, the episode had to be new (onset after ≥ 7 diarrhoea-free days), acute (onset within the previous 7 days), and fulfil at least one of the following criteria for moderate-to-severe diarrhoea: sunken eyes (confirmed by parent or caretaker as more than normal; loss of skin turgor (abdominal skin pinch with slow [≤ 2 s] or very slow [> 2 s] recoil); intravenous hydration administered or prescribed; dysentery (visible blood in loose stools); or admission to hospital with diarrhoea or dysentery (14) . For each child with MSD, at least one healthy control child (no story of diarrhea in the previous 7 days) was randomly selected from the neighborhood in which the case resided using the DSS database within 14 days of presentation of the index case. Controls were also matched by age and gender. After informing the child's representative of the objectives and characteristics of the study and obtaining their written informed consent, clinical and epidemiology data were obtained, anthropometric measurement performed and the mother or guardian of a child was given a stool container and instructions for sample collection. Case samples were collected within 12 hours of registration of the diarrheal episode, and control samples within 14 days after case enrolment. Once collected, samples were kept in a cool box until processed and history of taking antibiotics in the previous 4 hours was recorded. Each fecal specimen comprised a whole stool specimen (in screw top fecal specimen cups carried in Styrofoam boxes with cold packs), a fecal swab in Modified Cary Blair medium in a plastic screw top test tube, and a fecal swab in buffered glycerol saline in a screw top test tube (15).

Specimen processing for pathogen detection

Fecal specimens were plated on media for detection of bacterial pathogens according to standard methods (16). Bacterial agents (*Salmonella*, *Shigella*, *Campylobacter*, *Aeromonas*, and *Vibrio* spp) were detected using conventional culture techniques. Three putative *Escherichia coli* colonies from every stool were pooled and analysed by multiplex PCR that detect targets for enterotoxigenic (ETEC), entero aggregative (EAEC), enteropathogenic (EPEC), and entero haemorrhagic *E coli* (EHEC). The following gene targets defined 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), and EHEC (*eae* with *stx1*, *stx2*, or both, and without *bfpA*). Commercial immunoassays detected rotavirus (ELISA ProSpecT Rotavirus kit, Oxoid, Basingstoke, UK) and adenovirus (ProSpecT Adenovirus Microplate (Oxoid); adenovirus-positive samples were tested for enteric adenovirus serotypes 40 and 41 (Premier Adenoclone kit, Meridian Bioscience, Cincinnati, OH, USA). Norovirus (genotypes I and II), sapovirus, and astrovirus were detected using multiplex reverse transcriptase (RT) PCR. Individual commercial immunoassays (TechLab, Inc, Blacksburg, VA, USA) detected *Giardia lamblia*, *Entamoeba histolytica* and *Cryptosporidium* spp.

Statistical analysis

Analyses were conducted by using the STATA software (version 12.0) (StataCorp LP, College Station, TX, USA). Minimum community-based incidence rates between 2001 and 2012 were calculated referring cases to population denominators, establishing time at risk (child years at risk (CYAR)) inferred from the DSS census information. Negative binomial regression models were estimated to compare incidence rates between age-groups or calendar

years. Children did not contribute to the numerator/denominator for a period of 7 days after each episode of diarrhea or when they were outside the study area. For the case-control analysis, variable with missing value in its matched pair were not included and differences between matched pairs were evaluated by paired t-test or Sign test of matched pairs for continuous variables and by Exact McNemar significance probability test or Symmetry (asymptotic) test for categorical variables. Associations with MSD were estimated by conditional logistic regression, and multivariable models were estimated using a step-down process including all those variables with a percentage of missing or unmatched values less than 5% and with p-value < 0.2 in the crude analysis and adjusted for socio-demographic and nutritional variables, other pathogens and interactions between pathogens. Weighted attributable fractions (AF) of MSD (unadjusted/ adjusted), annual attributable cases and attributable incidences were calculated for all variables with a positive association with MSD. According to the study protocol, cases of MSD were included in approximately equal numbers each fortnight, regardless of the number of cases having visited health facilities within the DSS area. This was taken into account and weighted attributable fractions were also estimated, using weights defined as the inverse of the sampling fraction (number of eligible cases divided by the number of enrolled cases in each fortnight) (14). These weights were calculated separately for cases with and without dysentery, to avoid any bias from overrepresentation or underrepresentation of cases with dysentery. We combined data for two or more adjacent fortnights to have at least one case with dysentery and at least one case without dysentery in each time period. Unadjusted/adjusted and weighted/unweighted attributable fractions were calculated as proposed by Bruzzi et al (17). To estimate the burden of MSD, repeated surveys were conducted during the case-control study to find out the proportion of cases that usually goes to the health facilities within one week of onset of MSD (called r). We combined the data from these surveys and we weighted them by sampling

weights, based on the number of children in each age-sex stratum according to information from the DSS during each round (14). Then, we estimated the values of r (figure 1) and its variances for each age stratum by Kaplan Meier analysis. The annual number of cases of MSD in the population was calculated as the average number of eligible cases per year divided by r . The annual cases divided by the median of the population gave the MSD incidence rate. To calculate the number of cases and the incidence rates attributable to a specific pathogen, the total cases and incidence rates were multiplied by the pathogen's weighted and adjusted AF.

Ethical considerations

This study is part of the Global Enteric Multicenter Study (GEMS), a large multicenter study conducted in six other developing countries investigating the etiology and epidemiology of diarrheal disease in infants and young children. The overall protocol and informed consent were both approved by the National Bioethics Committee of Mozambique (CNBS), the ethics committee of the Hospital Clinic of Barcelona and the Institutional Review Board at the University of Maryland.

Results

Historical trends and burden of diarrheal disease

Yearly minimum community-based incidence rates (MCBIRs) of acute diarrheal hospital admissions during 2001-2012 are shown in figure 2. Throughout the decade MCBIRs have been highest in the 0-11 month's age group and lowest in the 24-59 months. All age groups have shown a steady decline that represents an 88% drop over the twelve years period in the older age group, a 77% in the 12-23 months and a 76% in the youngest group. The risk of acute diarrhea decreased with increasing age (12-23 vs. 0-11 months, IRR= 0.72, 95%CI: 0.67-0.77; 24-59 vs. 0-11 months, IRR= 0.10, 95%CI: 0.10-0.11; $p<0.001$). Point estimates of weighted annual incidence for MSD during 2007-2011 delivered from the surveillance and the case-control study were 9.85 episodes in infants (0-11 months), 7.73 in children aged 12-23 months and 2.10 per 100 CYAR in children aged 24-59 months.

The case control study

Figure 3 presents the study profile. During the 4-years study period, a total of 1696 children aged <5 years presented with MSD criteria and among these, 48% (816/1696) were not enrolled and 6% (96/1696) refused to participate. We finally recruited 784 children with MSD and 1545 matched children with no diarrhea. Because of the matched selection procedure, MSD cases were nearly identical to control children with respect to sex and age. The occurrence of criteria used for defining a MSD case for the enrolled children are presented in table 1. When we compared socio-demographic and health indicators among cases and controls, controls were more likely to present better hygienic behaviors (washing hands) in the three age groups. However, no strong evidence of an association between diarrhea morbidity with caretaker's formal education and household access to improved water or sanitation facilities could be found (data not shown).

We identified at least one enteropathogen in 666 (85%) children with MSD and in 1214 (76%) of the controls; and two or more agents in 376 (48%) cases and in 596 (37%) controls ($p < 0.001$). Table 2 summarizes the frequency of pathogens isolated in fecal samples in both the MSD and control groups. The etiologic agents detected more frequently included rotavirus, *G lamblia*, *Cryptosporidium*, EAEC aatA, and *E hystoliyca*, although there were differences across age groups. Crude and adjusted analyses of pathogens associated to MSD according to age group and consequent attributable fractions are shown in Table 3. Importantly, 53.97-64.38% of all MSD could not be attributable to any of the pathogens isolated. Rotavirus and *Cryptosporidium* were significantly associated with MSD in infants, while ETEC ST (ST only or ST/LT), *Shigella*, adenovirus 40/41 and rotavirus were associated to MSD in children aged 12-23 months. Our models could not confirm the association of any specific pathogen with MSD in the older age group. There was a negative interaction between Rotavirus and ETEC ST (ST only or ST/LT): OR= 0.01 (95% CI: 0.00-0.11) in children aged 12-23 months and paradoxically, *G lamblia* was consistently associated with a lower risk of MSD in all age groups.

Discussion

In this study we have documented the sharp decline throughout a period of over a decade in the incidence of diarrhea in Mozambican children. This probably reflects the general improvement in living conditions since the end of civil strife in the early 90's and the subsequent improvement in demographic, health and socio-economic status in children and the expansion and access to basic health care. During the same period, under-five mortality has decreased in the area from 135 to 91 per 1,000 live births and the infant mortality rate from 77 to 53 per 1,000 live births (Manhiça DSS communication). These are indeed massive health gains. However, diarrheal disease remains a major cause of morbidity in children aged less than five in Manhiça district as in most of the country. Thus, our findings reinforce the need to improve the implementation of diarrhea-specific control measures, particularly among children aged 0-23 months who presented the highest risk. With the identification of the pathogens independently associated with MSD and their respective pathogen-specific AF, we estimated that 35.62-46.03% of MSD could be reduced with specific interventions against those particular pathogens. This implies however that more than half (53.97-64.38%) of all MSD could not be attributable to any of the pathogens isolated. Incidences in the case-control study appear higher than calculated MCBIRs, due to the fact that MCBIR correspond to diarrhea cases seeking care at the hospital, while MSD incidences were calculated using all eligible MSD adjusted by $r(18)$ (thus total MSD regardless of health facility use).

The major finding of this study is the confirmation of rotavirus as the main cause of diarrhea in children aged less than five in Manhiça district. Rotavirus accounted for more than a third of MSD cases in infants, and its incidence rates markedly exceeded those of others pathogens. Diarrhea caused by rotavirus remains frequent and imposes a substantial burden on the health system, so a vaccine against rotavirus could hasten the decline of diarrheal disease morbidity.

The high prevalence found in this study is in close accordance with that of various regions of Sub-Saharan Africa, where rotavirus has been described as the leading cause of diarrheal disease (19). Contrariwise, in a study conducted in Manhiça more than 10 years ago (7), rotavirus was only detected in 1% of symptomatic children younger than 5 years of age, and this was probably due to the low sensitivity and specificity of the used assays. In the present study, adenovirus 40/41 was comparatively rare; nevertheless it does not necessarily exclude this pathogen as an important cause of diarrhea in children, particularly because adenovirus 40/41 remained significantly associated to MSD in children aged 12-23 months.

Cryptosporidium, a pathogen described to be the organism most commonly isolated in HIV-positive patients presenting with diarrhea was also frequently isolated (20). Unfortunately, data on HIV/AIDS co-infection, highly prevalent in the Manhiça district already at the time of the study were not collected, and therefore the direct impact of concomitant infections with HIV/AIDS cannot be analyzed. However, assuming the high HIV prevalence in the area, the natural history of HIV with the risk of disease progression being inversely correlated with the age of the child (greater risk of progression in the first year of life) (21) and the fact that *cryptosporidium* was only associated to MSD in the first year of life, there is evidence to suggest that HIV infection may be implicated as a huge contributor to diarrheal severity in the area and so that measures to reduce exposure to *cryptosporidium* and HIV must be improved.

Shigella, which is known to determine outbreaks of diarrhea in various communities (22, 23), was just isolated in 44 (6%) patients with MSD. Its low isolation rate in children without diarrhea 3 (0%), confirms the high pathogenicity of this agent. *Shigella flexneri* was the most prevalent serotype isolated in our study (80%) and the most frequently isolated in previous studies in Manhiça district (24).

The occurrence of *Escherichia coli* ranging from 9-30% of the children, is higher than those firstly published in this district in the same age group (7). The most frequently *E. coli* pathotype found was EAEC aatA, however ETEC ST was alone one of the MSD contributors in the second year of life. The absence of EHEC strains is not surprising and neither is the lower frequency of *Salmonella* and *Campylobacter* found in our study. These data are consistent with those from other neighboring areas in which these microorganisms were isolated in less than 3% of children aged <5 years with diarrhea (7, 25, 26).

Vibrio cholerae 01 was isolated mainly in children aged above two with diarrhea. This age related pattern of pathogens is consistent with reports from studies conducted in other developing countries (27) and should be taken into account when considering appropriate management of childhood diarrhea in Mozambique. As no outbreak of cholera was reported in the area during the time of study, this finding supports the described changing epidemiology tendency of *V. cholerae* in recent years in several regions of Mozambique, from the “epidemic disease” to “endemic disease with epidemic peaks” as a result of the cumulative number of asymptomatic carriers at the end of each peak. *V. cholerae* was isolated in one child without diarrhea demonstrating the occurrence, albeit rare, of asymptomatic carriers in the region. WHO has recommended the use of antibiotic treatment in order to decrease the duration of disease and mortality, and control its transmission. However, and according to Mozambique’s Ministry of Health “the use of antibiotics for cholera treatment is expressly prohibited to avoid emergence of bacterial resistance to antimicrobials” (28).

Three findings concerning the appreciation of the role of the isolated pathogens as etiologic agents are noteworthy. First, *G lamblia* was consistently associated with a lower risk of MSD in all age groups. Second, there were high rates of positive stool samples with potential

enteropathogens in children without MSD. And finally, multiple co-infections were detected and one of them was negatively associated with the occurrence of MSD. The characteristics of the pathogens (pathogenicity, duration of excretion and interaction with other pathogens), host and environmental factors are recognized to be the justifications for the above results (29), thus future studies should explore in greater depth the abovementioned factors when studying diarrheal etiology, to better understand pathogen causality.

While some of the strengths and limitations of the study have been discussed previously by Kotloff et al (14, 30), it seems pertinent to mention other methodological observations arising from the analysis of the strengths and weaknesses of this study that should be taken into account. First, almost half of children with MSD criteria were finally not enrolled in the study, mainly on account of impossibility of obtaining stool samples. This factor might have contributed to lower estimations of the proportion of MSD attributable to specific pathogens. Second, due to the negative effect of the interaction between Rotavirus and ETEC ST (ST only or ST/LT) in children aged 12-23 months, AF of rotavirus become negative despite it was associated to an increased odd of MSD. Finally, mild-diarrhea cases (an early stage of MSD) were not investigated, and could contribute to provide adequate and timely preventive interventions. Irrespective of these limitations, the study constitutes a comprehensive survey of the impact of diarrheal disease and pathogens causing MSD among children aged 0-59 months that gives an overall adequate picture of the current situation of diarrheal disease in a typical rural Mozambican area.

In conclusion, despite the predominant decreasing trend, diarrheal diseases remain a major cause of morbidity mainly among children aged less than two in rural Mozambique. Rotavirus, *cryptosporidium*, *Shigella*, ETEC ST and Adenovirus 40/41 were the most

important causes of MSD. Thus, well-known preventive strategies including accelerating the introduction of rotavirus vaccine should be promoted on a wider scale to reduce the current diarrheal diseases burden. High rates of positive stool samples with potential enteropathogens in children without diarrhea and multiple co-infections were frequently observed, underlining the difficulties of determining the cause of an episode of MSD. Thus, the role of the isolated pathogens as etiologic agents and the impact of concomitant HIV/AIDS infection need to be further investigated among this population.

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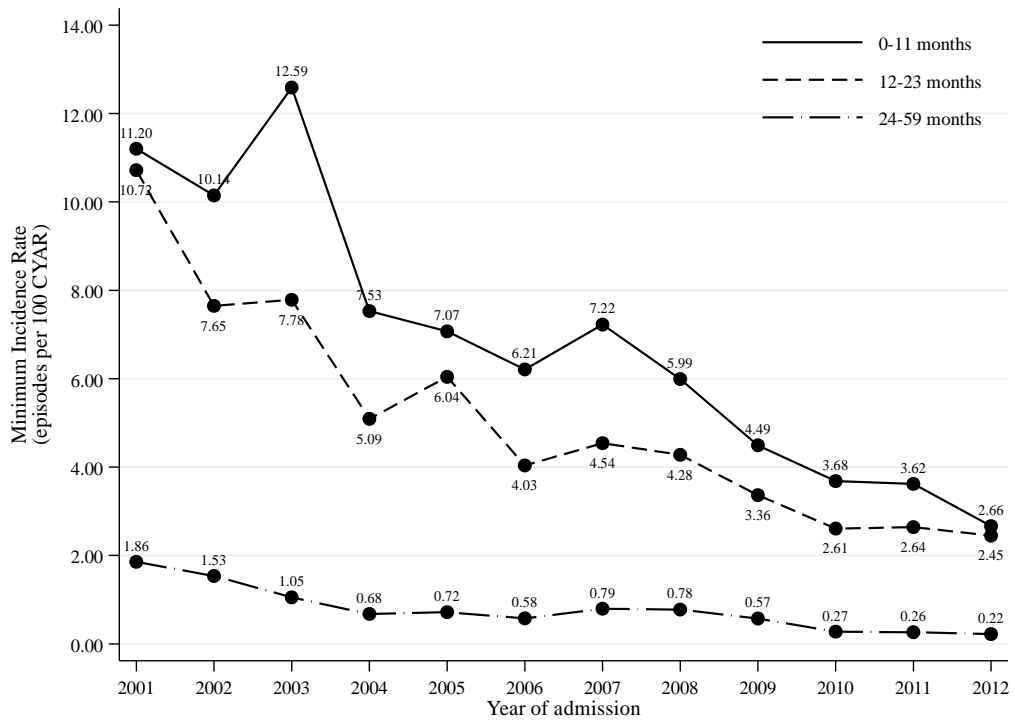
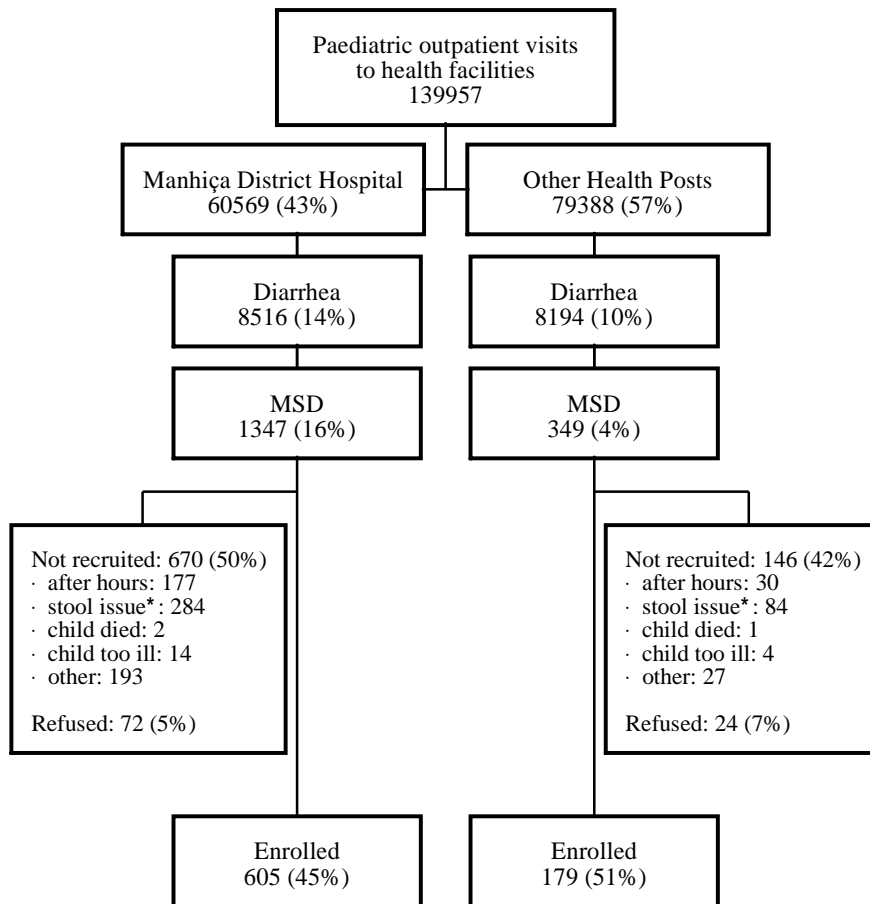


Figure 2: Minimum Community-based Incidence rate trends of hospitalized acute diarrhea episodes according to age during 2001-2012



* Stool issues include no specimen, insufficient specimen and improperly handled specimen.

Figure 3: Study profile showing number of patients and reason for not recruiting (December 2007- November 2011)

Table 1: Baseline characteristics of cases and controls included in the case control study

Characteristics	0-11 months	12-23 months	24-59 months
Number of enrolled children			
Case	431	233	120
Control	861	502	232
Age in months: median (IQR)			
Case	7 (5-9)	16 (14-19)	31 (27-37)
Control	7 (5-9)	16 (13-19)	30 (27-37)
Male sex: n (%)			
Case	259 (60)	132 (60)	69 (57)
Control	517 (60)	302 (60)	149 (64)
MSD criteria n (%)			
Hospitalization with diarrhea/dysentery	300 (70)	155 (67)	57 (48)
Sunken eyes	243 (56)	111 (48)	48 (40)
Receive intravenous fluids	145 (34)	100 (43)	39 (32)
Wrinkled skin	223 (52)	57 (24)	20 (17)
Dysentery	33 (8)	44 (19)	52 (43)

Denominator: number of cases with MSD

IQR (Interquartile range)

Table 2: Frequency of pathogens in stool samples of children with moderate-to-severe diarrhea and the control group

Pathogens	0-11 months			12-23 months			24-59 months		
	Cases	Controls	P	Cases	Controls	P	Cases	Controls	P
	N=431	N=861		N=233	N=502		N=120	N=232	
	n (%)	n (%)		n (%)	n (%)		n (%)	n (%)	
Protozoa									
<i>G. lamblia</i>	41 (10)	152 (18)	<0.001	64 (28)	228 (46)	<0.001	42 (35)	115 (50)	0.020
<i>Cryptosporidium</i>	84 (20)	86 (10)	<0.001	44 (19)	46 (9)	<0.001	11 (9)	18 (8)	0.229
<i>E. histolytica</i>	39 (9)	79 (9)	0.607	26 (11)	52 (10)	0.837	15 (12)	28 (12)	0.920
Viruses									
Rotavirus	182 (42)	139 (16)	<0.001	52 (22)	91 (18)	0.014	12 (10)	27 (12)	0.821
Adenovirus 40/41	9 (2)	8 (1)	0.087	6 (3)	5 (1)	0.150	-	-	-
Adenovirus non 40/41	4 (1)	22 (3)	0.134	4 (2)	7 (1)	0.389	0 (0)	6 (3)	-
Norovirus	19 (4)	38 (4)	0.578	10 (4)	25 (5)	0.286	4 (3)	12 (5)	0.109
Sapovirus	7 (2)	24 (3)	1.124	3 (1)	11 (2)	0.360	0 (0)	3 (1)	-
Astrovirus	7 (2)	11 (1)	0.600	6 (3)	10 (2)	0.477	1 (1)	4 (2)	0.477

Bacteria

ETEC ST (ST only or ST/LT)	20 (5)	19 (2)	0.040	29 (12)	16 (3)	<0.001	8 (7)	12 (5)	0.612
ETEC LT	12 (3)	58 (7)	0.005	17 (7)	32 (6)	0.409	3 (2)	7 (3)	0.818
EAEC aatA	95 (22)	184 (21)	0.563	27 (12)	29 (6)	0.032	9 (8)	14 (6)	0.906
EAEC aaiC	32 (7)	42 (5)	0.249	18 (8)	37 (7)	0.889	11 (9)	12 (5)	0.261
EAEC aaiC/aatA	23 (5)	47 (5)	0.548	15 (6)	26 (5)	0.693	1 (1)	9 (4)	0.121
EPEC typical	43 (10)	67 (8)	0.277	17 (7)	35 (7)	1.000	5 (4)	13 (6)	0.441
EPEC atypical	6 (1)	19 (2)	0.345	1 (0)	11 (2)	0.170	2 (2)	4 (2)	1.000
<i>Shigella</i>	6 (1)	1 (0)	1.000	18 (8)	2(0)	<0.001	20 (17)	0 (0)	-
<i>Salmonella non-Typhi</i>	6 (1)	6 (1)	0.115	2 (1)	0 (0)	1.000	-	-	-
<i>Vibrio cholerae 01</i>	-	-	-	4 (2)	1 (0)	0.145	9 (8)	0 (0)	1.000
<i>Aeromonas</i>	5 (1)	0.065	0.065	1 (0)	0 (0)	1.000	0 (0)	1 (0)	1.000
<i>Campylobacter</i>	24 (6)	0.339	0.339	9 (4)	14 (3)	0.289	0 (0)	2 (1)	-

P value not estimated due to no observation in the case group

Table 3: Crude and multivariate analysis, weighted attributable fractions and incidence of pathogens significantly associated with moderate-to-severe diarrhea

	Unadjusted		Adjusted ¹		Incidence rate per 100
	OR (95%IC)	AF (95%IC)	OR (95%IC)	AF (95%IC)	CYAR (95%IC)
0-11 months					
MSD total	-	-	-	-	9.85 (8.41-11.30)
MSD-attributable	-	-	-	46.03 (41.79-50.26)	4.54 (3.75-5.32)
Rotavirus	5.35 (3.87-7.38)	33.27 (30.80-35.74)	6.00 (3.65-9.87)	34.75 (31.30-38.20)	3.42 (2.82-4.03)
<i>Cryptosporidium</i>	2.62 (1.83-3.74)	12.78 (9.96-15.60)	3.67 (2.06-6.54)	15.26 (11.96-18.56)	1.56 (1.15-1.97)
12-23 months					
MSD total	-	-	-	-	7.73 (6.32-9.15)
MSD-attributable	-	-	-	35.62 (29.14-42.10)	2.75 (2.04-3.47)
<i>Shigella</i>	20.82 (4.78-90.70)	8.42 (7.79-9.04)	19.79 (3.33-117.67)	8.65 (7.83-9.57)	0.67 (0.53-0.81)
ETEC ST (ST only or ST/LT)	4.63 (2.37-9.06)	9.91 (8.08-11.74)	33.50 (5.80-193.54)	3.68 (-15.37-22.73) ²	0.28 (-1.19-1.76) ²
Adenovirus 40/41	2.46 (0.72-8.39)	1.69 (0.27-3.12)	14.05 (2.06-95.84)	2.73 (2.33-3.13)	0.21 (0.16-0.26)
Rotavirus	1.68 (1.11-2.55)	-	3.30 (1.60-6.79)	-	-

24-59 months

MSD total	-	-	-	-	2.10 (1.45-2.76)
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(1): Adjusted for socio-demographic and nutritional variables, other pathogens and pathogen's interaction

(2): Negative values at lower limit are due to a negative interaction between *ETEC ST* and *Rotavirus* in the multivariate model

AF: attributable fraction; CYAR: child years at risk; OR: odd ration

8.3 Article 3: Diarrheal disease in rural Mozambique. Part II: Risk factors of moderate-to-severe diarrhea among children aged 0-59 months

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Abstract

Background

The last decade has witnessed a dramatic decrease in the burden of diarrheal disease. However, it remains one of the leading causes of disease and death among children under 5 years in Mozambique. Improved understanding of the risk factors associated with the occurrence of diarrheal disease may help explain recent trends, as well as support and target appropriate interventions.

Methods

A case-control study was conducted in Manhiça district involving 784 children with diarrhea and 1545 controls aged 0-59 months. Clinical, epidemiological and anthropometric risk factors associated with moderate-to-severe diarrhea (MSD) were examined.

Results

Having a caretaker who was not the mother but not its educational level and giving stored water were independent risk factors for MSD. On the other hand, regular washing hands particularly after handling animals or before preparing baby's food, and having facilities to dispose child's stool were protective factors. Risk of MSD was not found to be strongly associated with economic indicators of the households.

Conclusions

While communities undergo socio-economic development, it may be possible to accelerate reduction of the burden of diarrheal disease through simple, inexpensive and cross-cutting interventions which promote healthy behavior such as hand washing, safe human waste disposal and breastfeeding practice.

Introduction

Globally, children aged less than five years experience, on average, 3.2 episodes of diarrhea every year [1]. Such an enormous burden is translated into 800,000 annual child deaths from diarrhea in this age group, representing up to 11% of the total burden of pediatric deaths. The impact of diarrheal diseases is particularly blatant in Sub-Saharan Africa and Southeast Asia accounting for more than 80% of all deaths [2].

Over the last decade, several reports have identified poor socioeconomic conditions, such as lack of access to safe water and sanitation, poor hygiene practices and unsafe human waste disposal as factors associated with diarrheal disease [3, 4]. Therefore, hygienic habits and improved water quality, exclusive breastfeeding during the first six months of life followed with a progressive introduction of other foods during the first 2 years of life have been recommended to reverse the intolerable toll that diarrhea poses in the health of children [5, 6]. In addition to these actions to prevent exposure to diarrhogenic habits, the administration of vaccines is likely to enhance an individual's ability to resist infections when exposed [7].

In Mozambique, a Southern sub-Saharan African country, incidence of diarrheal disease in children has dropped by about 80% over the last decade (T. Nhampossa et al, submitted). This drop would be generally attributable to improved socio-economic conditions but detailed understanding of risk factors that may have influenced that trend are still lacking. Diarrheal diseases still carry a high burden of morbidity and mortality, especially among younger children [8-11]. Thus, with the purpose of guiding public health policy and targeting appropriate interventions there is a compelling need to identify risk factors associated to diarrheal disease, particularly for moderate-to-severe diarrhea (MSD) a potential life-threatening condition.

This is the first study that simultaneously investigated environmental factors, primary caretaker's characteristics, the management of the illness and the microbiological etiology of MSD in Manhiça, a rural area of Southern Mozambique. In this country, approximately 65% of the population lives in rural settings [12] and are at similar risk of having diarrhea. We hereby present an analysis of the risk factors associated to MSD among all patients <5 years of age included as part of a larger, matched, case-control study on the etiology and epidemiology of diarrheal diseases conducted between the years 2007-2011 in Manhiça district, as part of the Global Enteric Multicenter Study (GEMS) [13].

Methods

Study area and population

The study area is located in Manhiça, Maputo Province, in Southern Mozambique. The Manhiça Health Research Center (CISM) runs a Demographic Surveillance System in the area [14] and a morbidity surveillance system at Manhiça District Hospital and at five other rural health posts. [15]. A detailed description of these and of the study area can be found in the companion article (T. Nhampossa et al, submitted).

Study design

Cases of moderate-to-severe-diarrhea and matched community controls were studied between December 2007 and November 2011. Case inclusion criteria included: age younger than 5 years, not currently enrolled as a case, seeking care at a sentinel health center belonging to the DSS area with an episode of acute diarrhea (not more than 7 days duration) and qualifying in intensity as “moderate-to-severe” (see below for definitions). For each child with MSD, at least one healthy control child (no story of diarrhea in the previous 7 days) was randomly selected from the neighborhood in which the case resided using the DSS database within 14 days of presentation of the index case. Controls were also matched by age and gender. After informing the child's representative of the objectives and characteristics of the study and obtaining their written informed consent, the following data were recorded at the time of the query: demographic, socioeconomic status, breastfeeding type, water and sanitation environmental and anthropometric measurement (weight, height and MUAC). We conducted a follow-up visit about 60 days after enrolment to assess the child's vital status, capture interim medical events, and repeat anthropometric measurements.

Statistical analysis

Analyses were performed using the STATA software (version 12.0) (StataCorp LP, College Station, TX, USA). Variables with missing value in their matched pair were not included and differences between matched pairs were evaluated by paired t-test or Sign test of matched pairs for continuous variables and by Exact McNemar significance probability test or Symmetry (asymptotic) test for categorical variables. Conditional logistic regression models were estimated to evaluate associations with MSD and multivariable models were estimated using a step-down process including all those variables with a percentage of missing or unmatched values less than 5% and with p-value < 0.2 in the crude models.

Definitions

Following the WHO definition, diarrhea was defined as the increase in depositional rate (≥ 3 times/day) with a decrease in stool consistency [16]. The presence of at least one of the following criteria, confirmed by trained clinical officers or doctors, defined an episode as "moderate to severe diarrhea": (i) At least one of the following signs referring moderate to severe dehydration: sunken eyes significantly more than normal, wrinkled skin or intravenous fluids recommended at the health center, (ii) Dysentery (diarrhea with visible blood in stool) or (iii) Hospitalization with diarrhea or dysentery. Primary caretaker education was stratified in two groups: no formal education (no education or did not complete primary education) or some formal education (at least completed primary education). Socioeconomic status was determined by the type of the house floor, number of rooms used for sleeping, family composition and being owner of any of the following: agricultural land, functioning electricity; radio; fridge/freezer; telephone/mobile; bicycle; motorcycle; television; and finally car/truck. Access to improved water was defined according to the aggregate's use of the following types of water supply for drinking: piped water, public tap, borehole or pump,

protected well, protected spring or rainwater. Water sources did not include vendor-provided waters, bottled water, tanker trucks or unprotected wells and springs, rivers or ponds. Improved sanitation facilities for the aggregate included: connection to a public sewer or septic system, pour-flush latrine, simple pit latrine, or ventilated improved pit latrine. Unimproved sanitation facilities include public or shared latrine, open pit latrine, or bucket latrine.

Ethical considerations

This study is part of the Global Enteric Multicenter Study (GEMS), a large multicenter study conducted in six other developing countries investigating the etiology and epidemiology of diarrheal disease in infants and young children. The overall protocol and informed consent were both approved by the National Bioethics Committee of Mozambique (CNBS), the ethics committee of the Hospital Clínic of Barcelona and the Institutional Review Board at the University of Maryland.

Results

Socioeconomic and demographic characteristics

The characteristics of the 784 cases and 1595 controls enrolled in the study are shown in Table 1. Univariate analyses of the risk factors are presented in table 2. Because of the matched selection procedure, MSD cases were nearly identical to control children with respect to sex and age. While the vast majority (94% for cases vs. 97% for controls) of children had the mother as the primary caretaker, those children who were being looked after by someone else had a significantly higher risk of developing MSD. More than two thirds (76% vs. 73%) of the primary caretakers were on the group of lower educational level but this was not associated with an increased risk of MSD. The relation between MSD and socio-economic status was examined through the ownership of different variables including (cases vs. controls): agricultural land (83% vs. 90%), telephone/mobile (74% vs. 78%), cement floors in the house (67% vs. 71%), radio (44% vs. 46%) and electricity (22% vs. 27%). Among these, being owner of agricultural land was the only variable significantly associated with a decreased risk of MSD for children aged less than two. Just 1-2% of the households reported owning none of the socio-economic variables examined. Having animals in the compound, more frequently reported in cases compared to their controls, was only associated with MSD in children aged 12-23 months.

Water exposure

The main sources of water to the study population were public tap (36% for cases vs. 31% for controls) and borehole (25% for cases vs. 24% for controls) (figure 1). More than the lack of access *per se*, the lack of consistency or regular use of the supplies was found to be associated with increased risk of MSD. Most of the population, (90% of the caretakers) reported fetching water daily with a median of 5 (IQR= 4-7) and 6 (IQR= 4-8) trips per day for cases and

controls respectively. From this, 12% of the cases and 22% of the controls needed over 30 minutes to get to the water source. Only a small proportion (6-9%) of the caretakers reported that they used to treat drinking water and among these, the use of chlorine (55% for cases vs. 75% for controls) and boiling water (45% for cases vs. 25% for controls) were the most commonly used methods.

Latrine, waste-disposal and hygiene characteristics

Hand washing appears as a strong protective factor against MSD. With regard to waste disposal, only 2% of the households did not have any facility whatsoever and did not report sharing or using facilities from other households. Again somehow surprisingly, this was not associated with increased risk of MSD. Most households used traditional pit toilet, (about 90% of cases and 92% of controls) rather than improved facilities such as improved latrines. Somehow counter intuitively; use of improved facilities was not associated with reduced risk of MSD.

Breastfeeding and nutritional characteristics

While there was no difference between the cases and the controls with respect to breastfeeding practices in the youngest group, children aged 12-23 months, who were partially breastfed (OR=0.46, CI; 0.30-0.70) or exclusively breastfed (OR=0.82, CI; 0.51-1.33, $p < 0.001$) had a lower risk of MSD compared to those not breastfed. The mean height-for-age z-score among both cases and controls was considerably below the WHO reference for all age groups and, with one exception, deviated further from the reference at older ages (12-59 months), however, the effect on the risk of MSD was small and of borderline significance.

Multivariate analysis

Independent risk factors for MSD according to age group by multivariate analysis are shown in table 3. Having a caretaker who was not the mother proved a very large risk factor for MSD particularly for infants and young children, followed by giving stored water to the child. On the other hand, washing of hands habit, mainly after handling animals or before preparing baby's food were found to be protective factors for MSD in all age groups. Having facilities to dispose child's stool was again associated with a lower risk of the occurrence of MSD.

Discussion

In Manhiça District, possibly quite representative of Southern Mozambique, we have demonstrated unprecedented declines in diarrheal disease incidences over the last 10 years. In the absence of specific interventions, we tend to intuitively associate this, with an ill-defined concept of “socio-economic” development as the key driver for this decline. Therefore improved and detailed understanding of the determinants of diarrheal disease may explain recent trends, as well as indicate potential interventions to be developed and deployed. However, in this study with the exception of ownership of land, the “basket” of economic indicators used at the households level have not been associated with MSD risk, and have therefore failed to associate economic status and MSD risk.

A strong independent determinant for MSD risk was having as primary caretaker someone other than the mother. This result is not surprising as the role of the mother in terms of care giving is historically defined and extends beyond simply feeding [17]. In general, the dependence to a primary caregiver tends to decrease with increasing age, probably explaining why this variable was not associated to MSD among the older age group. Remarkably, we failed to identify the formal education level of the primary caretaker with the risk of MSD. This is in contrast with the literature that suggests that the primary caretaker's increasing level of education has been considered an indicator of knowledge or behavior in relation to child health, and thus a protective factor against MSD [18, 19].

In this rural area, traditional pit toilets (not improved facility) were the kind of facility most commonly used to dispose human fecal waste. Only 2% of all households did not have any toilet facility, and this was not found to be a risk factor for MSD. One would imagine that these households share facilities with other households. Toilet sharing creates unsanitary and

unkempt conditions, which provide conducive environments for vectors and pathogenic organisms associated with diarrhea infection, and also increases the possibility of transmitting pathogens from one infected household to others [20, 21]. As sharing facilities' habit was never reported, the small proportion of households without any facility could be practicing open defecation. On the other hand, children living in households using improved facility were at increased risk, and this may reflect incorrect use or insufficient hygiene measures. Thus, measures to encourage the proper use of latrines linked to hygiene behavior are probably critical to accelerate improvement in Manhiça District.

A small proportion (6-9%) of the caretakers reported that they used to treat drinking water. Nevertheless, regularly treating drinking water behavior was a significant risk factor for MSD in infants. In addition, access to improved water source (frequently reported) was not protectively associated with the occurrence of MSD. These results are striking and may suggest that, either treating drinking water is incorrectly performed, or that conservation methods employed are not safe (in fact giving stored water to a child increased the odds of MSD). It is also likely that asking directly about "do you usually treat drinking water at home?" led to a significant response bias that was possibly conditioned by the fact that the caretakers wanted to impress a good behavior related to water quality. While the confirmation of the different primary caretaker answers was done by interviewer's observation of sanitation facilities or water source during community controls enrollment, confirmation and record of chlorine result test was only performed in the follow-up visit and verification of the boiling water process was almost impossible. Therefore, our findings reinforce the need to improve the implementation of diarrhea control measures with emphases on monitoring of water quality and adjusting the levels of chlorination in water supply.

In sharp contrast to studies that have consistently shown that stunting is a significant risk factor for childhood diarrheal disease [22], we failed to demonstrate this association in the present study. Moreover, multivariate analysis revealed the protective effect of breastfeeding only for children aged 12-23 months. Overmatching due to the fact that case and controls were likely to share the same nutritional and breastfeeding conditions could explain the above results. However, the protective effect of breastfeeding may have also been affected by the high HIV/AIDS infection in Manhiça at the time of the study, given the poor implementation strategies to prevent mother to child HIV transmission. Unfortunately, data on HIV/AIDS co-infection were not routinely collected, during the study and therefore the direct impact of concomitant infections with HIV/AIDS cannot be analyzed. Assuming the reported high HIV prevalence in the area, future studies on the risk factors for diarrheal disease among children should further investigate the above associations and local practices related to breastfeeding patterns and the role of HIV infection to shed a light on their impact on diarrheal illness.

In conclusion, while communities undergo economic development, it may be possible to markedly accelerate the reduction of the burden of diarrhea disease through aggressive implementation of simple, inexpensive and cross-cutting interventions which promote healthy behavior such as hands washing, safe human waste disposal and breastfeeding practice. Nevertheless, we highlight that more extensive measures to reduce poverty and hunger and promote social inclusion, as well as a wider application of community education targeting the prevention of diarrheal disease in general are aspects that also need to be urgently considered by the national public health policy makers.

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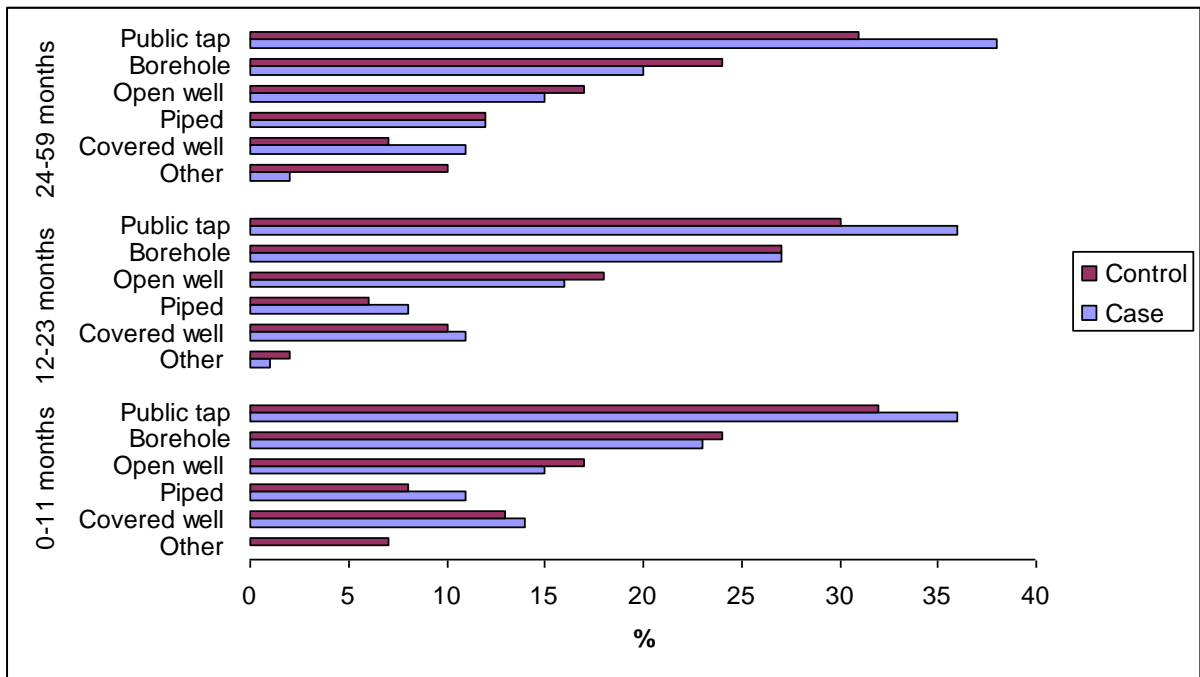


Figure 1: Main water sources among cases and controls included in the study according to age group

Table 1: Characteristics of the children included in the study

Variables	0-11 months		12-23 months		24-59 months	
	Cases n (%)	Controls n (%)	Cases n (%)	Controls n (%)	Cases n (%)	Controls n (%)
	N=431	N=861	N=233	N=502	N=120	N=232
Age in months: median (IQR)	7 (5-9)	7 (5-9)	16 (14-19)	16 (13-19)	31 (27-37)	30 (27-37)
Child sex (male)	259 (60)	517 (60)	132 (57)	302 (60)	69 (57)	149 (64)
Caretaker formal education						
No formal education	316 (74)	606 (71)	183 (79)	371 (75)	92 (77)	177 (77)
Some formal education	112 (26)	251 (29)	48 (21)	123 (25)	27 (23)	54 (23)
Family size: median (IQR)	6 (4-8)	7 (5-9)	6 (5-8)	6 (5-9)	6 (5-8)	7 (5-9)
Improved cooking fuel	6 (1)	3 (0)	0	0	0	2 (1)
Animals in compound	359 (83)	758 (88)	191 (82)	453 (90)	99 (82)	195 (84)
Main water source						
No improved water	71 (16)	146 (17)	39 (17)	92 (18)	21 (18)	39 (17)
Improved water	360 (84)	715 (83)	194 (83)	410 (82)	99 (82)	193 (83)

Fetch water daily	318 (91)	668 (91)	177 (89)	396 (88)	89 (90)	178 (90)
Give stored water to child	394 (91)	671 (78)	227 (97)	471 (94)	113 (93)	216 (93)
Treating water habit	51 (12)	57 (7)	11 (5)	25 (5)	7 (6)	9 (4)
Facility to dispose child's stool	208 (49)	511 (60)	187 (81)	472 (95)	115 (96)	228 (99)
Disposal of stool at the aggregate						
Flush/ Pour flush toilet	7 (2)	8 (1)	1 (0)	3 (0)	3 (3)	3 (3)
Ventilated improved pit	31 (7)	46 (5)	13 (6)	18 (4)	10 (8)	15 (6)
Traditional pit toilet	383 (89)	738 (92)	217 (93)	467 (93)	103 (86)	208 (90)
No facility	7 (2)	15 (1)	2 (1)	13 (2)	4 (3)	6 (3)
Washing of hands habit						
Before eating	406 (94)	817 (95)	204 (88)	482 (96)	111 (92)	219 (94)
Before cooking	265 (61)	731 (85)	135 (58)	449 (89)	71 (59)	193 (83)
Before preparing baby's food	113 (26)	599 (70)	71 (30)	368 (73)	31 (26)	147 (63)
After defecating	370 (86)	767 (89)	204 (88)	456 (91)	106 (88)	210 (91)
After handling animals	12 (3)	257 (30)	4 (2)	146 (29)	5 (4)	60 (26)

After cleaning child feces	107 (25)	495 (57)	51 (22)	261 (52)	23 (19)	10 (44)
Breastfeeding						
No	12 (3)	15 (2)	90 (39)	143 (28)	98 (82)	225 (97)
Partially	246 (57)	495 (57)	103 (44)	265 (53)	12 (10)	7 (3)
Exclusively	173 (40)	351 (41)	39 (17)	94 (19)	10 (8)	0
Height-for-age z-score: mean (SD)	-1.21 (1.42)	-1.09 (1.22)	1.75 (1.42)	-1.55 (1.18)	-1.55 (1.33)	-1.68 (1.21)

IQR (Interquartile range)

Table 2: Risk factors for moderate-to-severe diarrhea by univariate analysis

Variables	0-11 months			12-23 months			24-59 months		
	OR	95%CI	<i>P</i>	OR	95%CI	<i>P</i>	OR	95%CI	<i>P</i>
Child primary caretakers (not the mother)	4.81	1.91-12.01	<0.001	2.75	1.32-5.75	0.007	0.96	0.44-2.09	0.921
Caretakers with some formal education	0.93	0.70-1.24	0.632	0.80	0.53-1.20	0.280	1.03	0.58-1.84	0.922
Family size increasing	0.94	0.90-0.97	<0.001	0.97	0.92-1.02	0.228	0.91	0.84-0.99	0.027
Number of sleeping rooms	0.86	0.78-0.94	0.001	1.02	0.90-1.16	0.717	0.84	0.68-1.03	0.094
Owner of agricultural land	0.53	0.36-0.76	<0.001	0.60	0.38-0.96	0.033	0.46	0.20-1.09	0.079
Owner of car /truck	1.46	0.83-2.55	0.188	1.44	0.71-2.93	0.315	3.00	0.94-9.53	0.063
Animals in compound	0.76	0.54-1.08	0.126	0.56	0.35-0.88	0.012	1.07	0.50-1.95	0.820
Improved water (as main water source)	1.18	0.80-1.73	0.401	1.29	0.76-2.18	0.345	0.87	0.44-1.72	0.682
Water availability (not always/day)	3.16	2.40-4.17	<0.001	3.26	2.23-4.78	<0.001	2.31	1.37-3.89	0.002
Give store water to child	4.26	2.66-6.83	<0.001	2.90	0.95-8.85	0.061	1.95	0.52-7.34	0.324
Treating water habit	1.93	1.27-2.92	0.002	0.79	0.36-1.74	0.557	1.21	0.33-4.46	0.777
Facility to dispose child's stool	0.65	0.49-0.83	<0.001	0.24	0.14-0.41	<0.001	0.25	0.06-1.10	0.066

No improved facility for aggregate's stool	0.64	0.41-1.01	0.058	0.63	0.31-1.30	0.212	0.71	0.29-1.69	0.433
Washing of hands habit									
Before eating	0.91	0.54-1.54	0.737	0.29	0.16-0.55	<0.001	0.56	0.21-1.51	0.254
Before cooking	0.26	0.19-0.35	<0.001	0.11	0.07-0.18	<0.001	0.26	0.15-0.45	<0.001
Before prepare baby's food	0.12	0.08-0.16	<0.001	0.13	0.08-0.19	<0.001	0.14	0.08-0.27	<0.001
After defecating	0.77	0.53-1.11	0.159	0.74	0.44-1.25	0.257	0.84	0.40-1.75	0.636
After handling animals	0.04	0.02-0.09	<0.001	0.01	0.00-0.06	<0.001	∞	-	1.000
After cleaning child feces	0.19	0.14-0.26	<0.001	0.19	0.13-0.29	<0.001	0.18	0.09-0.35	<0.001
Breastfeeding									
No	1			1			1		
Partially	0.71	0.32-1.58		0.46	0.30-0.70		9.12	1.96-42.49	
Exclusively	0.82	0.63-1.08	0.227	0.82	0.51-1.33	<0.001	∞	-	0.019
Height-for-age z-score: mean (SD)	0.90	0.82-1.00	0.043	0.87	0.76-1.00	0.048	1.05	0.87-1.25	0.625

Table 3: Independent risk factors for moderate-to-severe diarrhea
by multivariate analysis

Variables	OR	95%CI	<i>P</i>
0-11 months			
Child primary caretakers (not the mother)	13.71	3.73-50.47	<0.001
Number of sleeping rooms	0.94	0.90-0.99	0.012
Owner of agricultural land	0.51	0.29-0.87	0.014
Owner of car/truck	2.44	1.02-5.79	0.044
Give stored water to child	7.46	3.83-14.55	<0.001
Treating water habit	2.05	1.12-3.75	0.020
No improved facility for aggregate's stool	0.43	0.22-0.85	0.016
Washing before cooking	0.35	0.24-0.51	<0.001
Washing before prepare baby's food	0.24	0.16-0.36	<0.001
Washing after handling animals	0.07	0.03-0.19	<0.001
Washing after cleaning child feces	0.57	0.38-0.86	0.009
12-23 months			
Child primary caretakers (not the mother)	3.77	1.11-12.77	0.033
Water availability (not always/day)	1.88	1.10-3.21	0.021
Give stored water to child	3.88	1.01-14.93	0.049
Facility to dispose child's stool	0.39	0.16-0.76	0.008
Washing hands before eating	0.39	0.16-0.94	0.035
Washing hands before cooking	0.12	0.06-0.24	<0.001

Washing hands before prepare baby's food	0.33	0.19-0.58	<0.001
Washing after handling animals	0.03	0.00-0.24	<0.001
Breastfeeding			
No	1		
Partially	0.43	0.23-0.83	
Exclusively	1.68	0.82-3.44	0.032
24-59 months			
Owner of agricultural land	0.32	0.10-0.99	0.049
Water availability (not always/day)	1.97	1.02-3.83	0.045
Facility to dispose child's stool	0.03	0.00-0.40	<0.001
Washing hands before cooking	0.42	0.21-0.85	0.016
Washing hands before prepare baby's food	0.21	0.10-0.43	<0.001
Washing after cleaning child feces	0.31	0.13-0.73	0.007

**8.4 Article 4: Healthcare Use and Attitudes Survey in cases of moderate-to-severe
diarrhea among children ages 0-59 months in the district of Manhica,
Southern Mozambique**

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Health Care Utilization and Attitudes Survey in Cases of Moderate-to-Severe Diarrhea among Children Ages 0–59 Months in the District of Manhiça, Southern Mozambique

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Abstract. In the predominantly rural Manhiça district, in southern Mozambique, diarrhea is one of the leading causes of death among children under 5 years. Caretakers randomly selected from the Demographic Surveillance Database were invited to participate in a community-based survey on use of healthcare services for gastroenteritis. Of those caretakers reporting an episode of diarrhea during the recall period, 65.2% in the first survey and 43.8% in the second survey reported seeking care at a health facility. Independent risk factors for seeking care in health facilities in the first survey included the presence of diarrhea with fever and not knowing any sign of dehydration; having a television at home was related with an independent decreased use of the health facilities. In the second survey, the use of health services was significantly associated with diarrhea with fever and vomiting. Establishment of continuous prospective monitoring allows accounting for changes in healthcare use that may occur because of seasonality or secular events.

INTRODUCTION

The World Health Organization (WHO) ranks diarrheal disease as the second most common cause of mortality among children under 5 years (0–59 months) of age in developing countries. Each year, about 1.35 million children die of diarrhea in this age group, representing up to 15% of the total burden of pediatric deaths.^{1,2} The distribution of these deaths caused by diarrheal diseases, however, is very unbalanced, and the poorest countries are the most affected. More than 80% of all deaths in children younger than 5 years occur in sub-Saharan Africa and Southeast Asia, primarily as a result of worse social and sanitation conditions and lack of access to adequate treatments and healthcare services.^{3–6}

Since the independence in 1975, the Government of Mozambique has tried to promote equitable access to basic health services through the continued expansion of the primary healthcare system and additionally, the elimination of healthcare fees for children under 5 years of age.^{7,8} However, despite increased availability of health resources, infant mortality rates remain unacceptably high; they are estimated at 138 of 1,000 live births, and they are related, in most cases, to easily preventable infectious diseases.^{9,10} In Mozambique, as usually occurs in most other sub-Saharan African countries, diarrheal disease carries a high burden of morbidity and mortality, especially among younger children. Thus, diarrhea is estimated to be the third leading cause of death (accounting for at least 10% of all mortality) among children ages 0–14 years in the city of Maputo, the capital and an urban environment.¹¹ In the district of Manhiça (predominantly rural), diarrhea is the third leading cause of hospital admission among children ages 0–14 years and the fourth leading cause of death among children between 12 and 59 months¹² according to verbal autopsies performed in the area.

In a country where up to 65% of the population lives in areas considered rural, many of these deaths occur at home and therefore, away from health centers or hospitals. This result is primarily because of structural problems, which lead to limited access to health systems and to a lesser extent, a lack of recognition by parents or primary caretakers of children of the symptoms associated with serious illness. Thus, according to data from the Mozambican Ministry of Health, 60% of the population (12 million of a total of 20 million) has no access to the health system; this inaccessibility is defined as living 20 miles or farther away from any health facility.^{13,14} It, therefore, seems appealing to try to understand in greater depth the functioning of these health systems and the constraints associated with their use. This understanding is even more important in the case of diarrheal diseases in particular, because they often are considered at the population level as trivial illnesses that are not serious or do not require specialized care, despite their high associated morbidity and mortality in developing countries. Understanding the challenges related to access to health systems would be useful for the development of policies and programs designed to counterbalance such barriers, encourage equity in care, promote a better use of the available services, and ultimately, improve their quality, not only to improve their use by children but also, the general population.

The main objective of this study was to assess the perceptions and attitudes of primary caretakers in the community about diarrheal disease and its associated danger and also, determine what conditions are associated in a rural area like Manhiça with the use of healthcare services in children under 5 years of age with diarrheal disease.

MATERIALS AND METHODS

Study area and population. The study was based on two community surveys conducted in the District of Manhiça, a rural area located 80 km from the capital of Mozambique, Maputo (Figure 1). The first cross-sectional survey took place between May 8 and June 28 of 2007. The second survey

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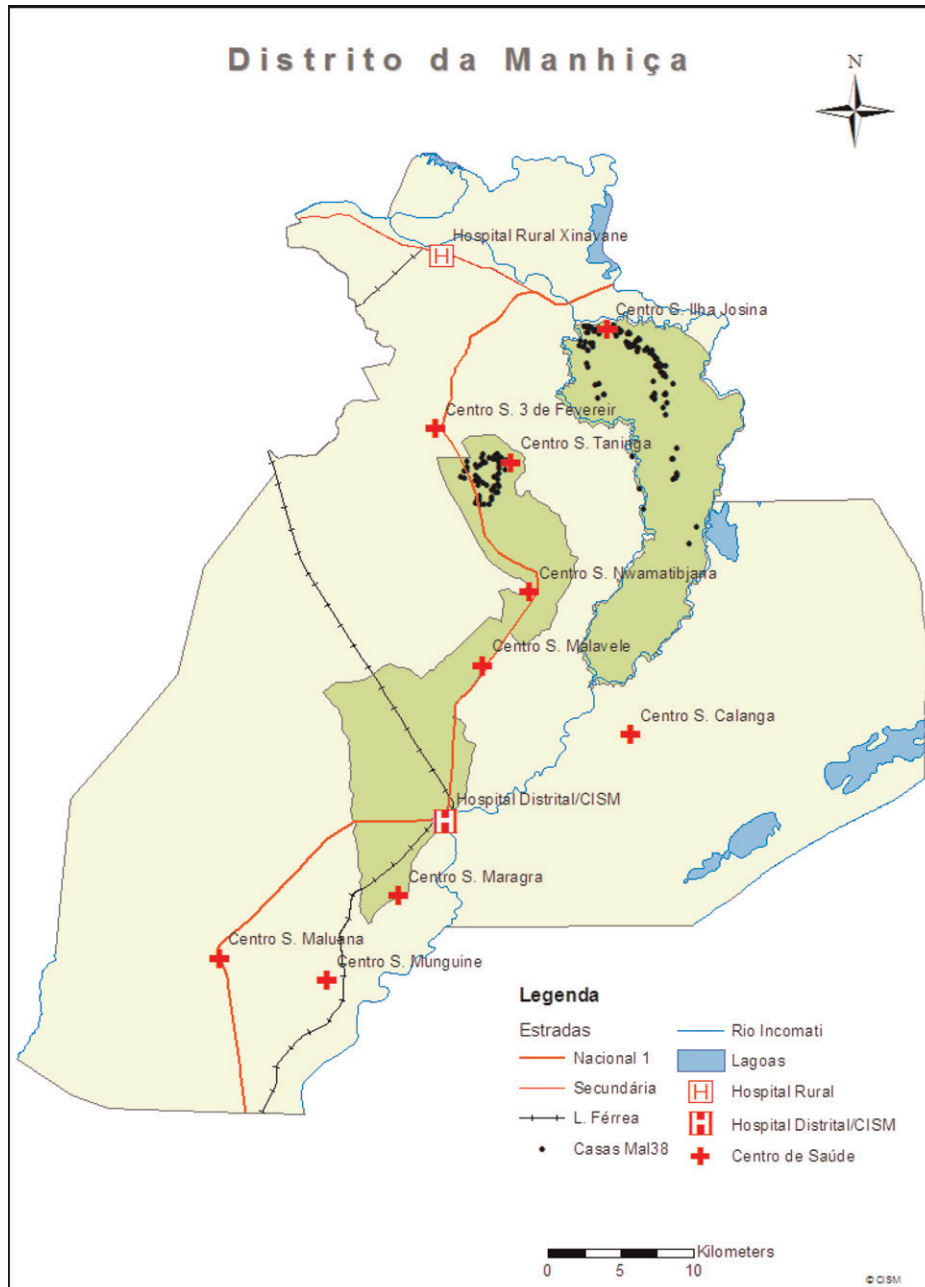


FIGURE 1. Manhica study area.

included a series of four repeated cross-sectional assessments that took place between February 16, 2009 and December 30, 2010 and was conducted to account for changes in healthcare use that may occur because of seasonality (e.g., flooding, harvest season, and holidays) or secular events (e.g., elections and political unrest). In Manhica, the climate is subtropical, with two distinct seasons: a warm and rainy season, usually spanning from November to April, and a generally cooler and drier season during the rest of the year. The average annual temperature ranges from 22°C to 24°C, and annual rainfall ranges from 600 to 1,000 mm. No droughts or floods occurred during the study period. Malaria transmission, mainly caused by *Plasmodium falciparum*, is perennial, with substantial seasonality and moderate intensity.¹⁵ At the time of the study,

malaria in Manhica accounted for one-third of all outpatient visits, one-half of the pediatric admissions, and 19% of all in-hospital pediatric deaths.^{16,17} Human immunodeficiency virus (HIV) prevalence in the district is very high¹⁸; in 2007, the prevalence of HIV among 646 hospitalized children included in a pneumonia study was 25%.¹⁹ The Manhica district has about 150,000 inhabitants. The Centro de Investigação em Saúde da Manhica (CISM) has run a demographic surveillance system (DSS) within this district since 1996, involving intensive and regular monitoring of a population of about 80,000 inhabitants in an area of around 500 km². About one-fifth (19%) of the study area inhabitants are children < 5 years of age.^{20,21} The first of the two surveys was restricted to a smaller area within the DSS, covering 100 km²

with 48,200 people, of which 17% (8,192) were children younger than 5 years of age.

Study design. Many of these study methods were adapted from the Generic Protocols for: I) Hospital-Based Surveillance to Estimate the Burden of Rotavirus Gastroenteritis in Children and II) a Community-Based Survey on Utilization of Health Care Services for Gastroenteritis in Children from the WHO.²² Data collection was performed through interviews conducted with primary caretakers of children ages 0–59 months living in the District of Manhica. For the first survey, 1,140 children were randomly selected from the DSS database and stratified into three age groups as follows: 400 children ages 0–11 months (a group larger than the rest because of the assumed higher difficulty for locating and recruiting children in this age group) and 370 children between 12–23 and 24–59 months. For the second survey, random sampling from the DSS occurred periodically to prevent a large number of children from ageing out of age strata. Children were not included in case of change of residence, death, migration, difficulties of finding the child's primary caretakers after three attempts, or if the child's age was confirmed to be older than 5 years at the time of the visit. In such cases, new candidates were included to complete the necessary numbers. A total of 1,289 households were visited during the first survey, and 3,601 households were visited in the four rounds of the second survey.

Study questionnaire. A standardized questionnaire was used for each child contacted. The data collected through 60 questions included information about household and family composition, number of other children under the responsibility of the primary caregiver, the time of onset and clinical symptoms of the last episode of diarrheal disease (as described by primary caretakers), and the practices and attitudes of use of health services in the same episode. Distance from the health center as well as topologic barriers of healthcare use were also calculated and collected. Finally, information on the perception of respondents about the risk of diarrheal diseases in children and the importance of developing vaccines and other interventions against this disease were also collected. In the absence of episodes of diarrhea within 14 days before the interview, mothers or caretakers were also asked about their likely use of health centers should their children hypothetically develop diarrhea. For the second survey, the questionnaire was simplified to clinical spectrum, and certain questions were removed (socioeconomic status and information on the perception of respondents about the risk of diarrheal diseases in children).

Statistical analysis. The analysis is based on the Global Enterics Multicenter Study (GEMS) Protocol regarding Healthcare Utilization and Attitudes Survey (HUAS). Briefly, analysis of data was performed using the Stata/SE software version 12.0 and its suite of survey data commands to account for stratified sample design and sampling weights. The survey was designed as a stratified random sample, with sex, age, and round (only for the second survey) as stratification factors. Sampling weights were constructed for each survey according to the DSS at CISM and applied to the sample data to produce accurate weighted population estimates. For each survey, we estimated the 2-week period prevalence of any diarrhea, moderate-to-severe diarrhea (MSD), proportion of diarrhea cases seeking care outside the home, and proportion of MSD cases seeking care at one of the designated GEMS case-control

study sentinel health facilities; 95% confidence intervals (CIs) were calculated through Jackknife variance estimation.²³ The χ^2 test was used for differences in proportions, and linear regression models were estimated to compare means. Multivariate logistic regression was used for identifying the factors independently associated with healthcare-seeking behavior at a study health facility among children who had diarrhea in the last 2 weeks. The model estimated using a backward-stepwise procedure for selection of variables, with a removal criterion of $P > 0.05$ by Wald test. Variables used to estimate the multivariate model were all those variables that had a P value < 0.10 in the crude association and did not have empty cells by doing a cross-tabulation with the outcome variable.

Definitions. Using the WHO protocol, diarrhea was defined as an increased frequency and volume and decreased consistency of stool from the norm.²⁴ The presence of at least one of the following criteria, when reported by mothers or primary caretakers of children with diarrhea, defined an episode as MSD: (1) at least one of the following signs indicating MSD: sunken eyes significantly more than normal, wrinkled skin, or intravenous fluids administered at the health center (as referred by the mother in the interview), (2) dysentery (diarrhea with visible blood in stool), or (3) hospitalization for diarrhea or dysentery. Primary caretaker education was stratified in two groups: no formal education (no education or did not complete primary education) or some formal education (at least completed primary education).

Ethical considerations. This study is part of a larger multicenter study conducted in six other developing countries investigating the etiology and epidemiology of diarrheal disease in infants and young children. The overall protocol and informed consent (obtained from the parents or legal guardians of minors) were both approved by the National Bioethics Committee of Mozambique (CNBS), the ethics committee of the Hospital Clinic of Barcelona, and The Institutional Review Board (IRB) of the University of Maryland, Baltimore, MD.

RESULTS

First survey. A total of 1,059 households was included in the first survey (Table 1). Of these children, 400 children were aged 0–11 months, 319 were aged 12–23 months, and 340 were aged 24–59 months, representing 16%, 24%, and 60% of the DSS population, respectively. The male/female ratio of children included in the survey was 1:1. The interview respondent was the mother in 851 (77%) of households. Almost two-thirds (69%) of the primary caretakers did not complete primary school education. The vast majority (84%) of children lived in houses with cement floors. The mean number of people per family aggregate was seven, and the mean number of compartments in the household used to sleep was two. Almost 90% (958) of the caretakers would reach the hospital on foot, and among these caretakers, 449 (46%) needed over 30 minutes to get there. Ownership within aggregates of different variables defining the level of socioeconomic status included: telephone/mobile (60%), radio (50%), electricity (20%), bicycle (19%), television (18%), refrigerator/freezer (10%), and car/truck (4%). One-fourth of the interviewed caregivers (25%) reported owning none of the above.

Diarrheal episode. Of 1,062 children selected for inclusion, 67 caregivers (representing 4% of the DSS population)

TABLE 1
Characteristics of the children included in the two surveys

Variables	1° Survey <i>n</i> (%)		2° Survey <i>n</i> (%)	
	Enrolled (<i>N</i> = 1,059)	DSS estimated population (<i>N</i> = 7,482)	Enrolled (<i>N</i> = 2,854)	DSS estimated population (<i>N</i> = 15,369)
Age group (months)				
0–11	400 (38)	1,207 (16)	880 (31)	3,232 (21)
12–23	319 (30)	1,791 (24)	973 (34)	3,125 (20)
24–59	340 (32)	4,484 (60)	1,001 (35)	9,013 (59)
Sex				
Male	547 (52)	3,783 (51)	1,448 (51)	7,821 (51)
Female	512 (48)	3,699 (49)	1,406 (49)	7,548 (49)
Total diarrhoea	67 (6)	321 (4)	246 (9)	1,027 (7)
Diarrhoea with*				
Mucus/pus	37 (55)	172 (54)	–	–
Fever	24 (36)	109 (34)	98 (40)	428 (42)
Thirst	26 (39)	128 (40)	85 (35)	360 (36)
Rice watery stool	9 (13)	57 (18)	108 (45)	444 (44)
Sunken eyes	16 (24)	62 (19)	74 (30)	305 (30)
Vomits	11 (16)	42 (13)	78 (32)	327 (32)
Unable to drink	–	–	72 (30)	297 (29)
Lethargy/unconscious	16 (24)	64 (20)	12 (5)	53 (5)
Irritable/less play	–	–	59 (24)	250 (24)
Blood in stool	4 (6)	17 (5)	18 (7)	90 (9)
Wrinkled skin	7 (10)	23 (7)	6 (2)	26 (3)
Seek care outside home*	44 (66)	222 (69)	125 (51)	521 (51)
Sources of care seeking outside home†				
Healthcare use	41 (93)	208 (94)	109 (87)	449 (86)
Pharmacy	3 (7)	22 (10)	3 (2)	11 (2)
Unlicensed practitioner	0	0	2 (2)	13 (2)
Bought medicines	1 (2)	5 (2)	3 (2)	16 (3)
Traditional healer	1 (2)	3 (1)	6 (5)	21 (4)
Health use with age* (months)				
0–11	20 (59)	60 (59)	51 (46)	187 (46)
12–23	15 (60)	76 (60)	45 (44)	144 (44)
24–59	6 (75)	73 (79)	13 (41)	118 (41)

*Denominator: number of children with diarrhoea.

†Denominator: number of children who sought care outside home.

reported at least one episode of diarrhoea during the 2 weeks before the interview. Of these 67 episodes, 21 (25%) were considered MSD. The mean duration of a diarrhoea episode was 4 days, and 57 (85%) of the children had a maximum number of three to six loose stools per day (Figure 2). The most commonly reported symptoms during the diarrhoeal episodes were: mucus/pus in stool (54%), intense thirst (40%), fever (34%), lethargy (20%), and sunken eyes (19%). The risk of diarrhoea decreased with increasing age (12–23 versus 0–11 months, odds ratio [OR] = 0.82, 95% CI = 0.48–1.42; 24–59 versus 12–23 months, OR = 0.29, 95% CI = 0.12–0.65; $P = 0.002$). There were no significant differences in symptoms according to age groups.

Attitudes and perceptions of diarrhoeal illness and healthcare-seeking behavior. Mothers or primary caretakers were asked about the main factors that make a diarrhoeal episode severe, the defining manifestations of dehydration, and the type of preventive measures useful against diarrhoea. The vast majority of the caretakers identified findings such as blood in stool (97%), stool increased frequency or decreased consistency (96%), vomiting (95%), rice watery stools (94%), or presence of dehydration (93%) as markers of severity accompanying a diarrhoeal episode. When enquired regarding defining manifestations of dehydration, the most common responses were the presence of sunken eyes (43%), thirst (43%), wrinkled skin (34%), decreased urinary frequency (33%), and lethargy (29%). Washing hands (45%), clean food or water (39%), and proper disposal of human waste (26%)

were the most commonly identified measures for preventing diarrhoea. There were no significant differences in the prevalence of reported diarrhoea according to the knowledge of markers of dehydration, severity, or measures to prevent diarrhoea. The vast majority of primary caretakers (98%) reported that vaccines are important for child's health and that they would be willing to use them, if available, to prevent any kind of diarrhoea. Most parents (85%) reported no problems in accessing health systems, but among those parents stating the contrary, the most frequent included the presence of long lines at the hospital (8%) and the lack of transportation to get there (7%).

Care-seeking behavior outside the home and healthcare-seeking behavior. Of those caretakers reporting diarrhoea during the recall period, 41 (65.2%, 95% CI = 51.9–78.4) of the primary caretakers reported seeking care at sentinel health facilities; this proportion increased to 85.9% (95% CI = 69.6–102.1) among those caretakers with children with MSD. Sentinel health facilities were the main sources of care seeking outside the home for diarrhoeal disease. Other sources of care seeking outside the home included going to the pharmacy (3; 10%), directly buying medicines at shop/market (1; 2%), or using a traditional healer (1; 1%). Mothers made the decision to go to the hospital in 84% of the households, and about 39% of the diarrhoea cases sought care in the first day of diarrhoea. Healthcare use rose with increasing age: 58.8% (95% CI = 41.8–75.9) for children ages 0–11 months, 60.0% (95% CI = 39.8–80.2) for children ages 12–23 months,

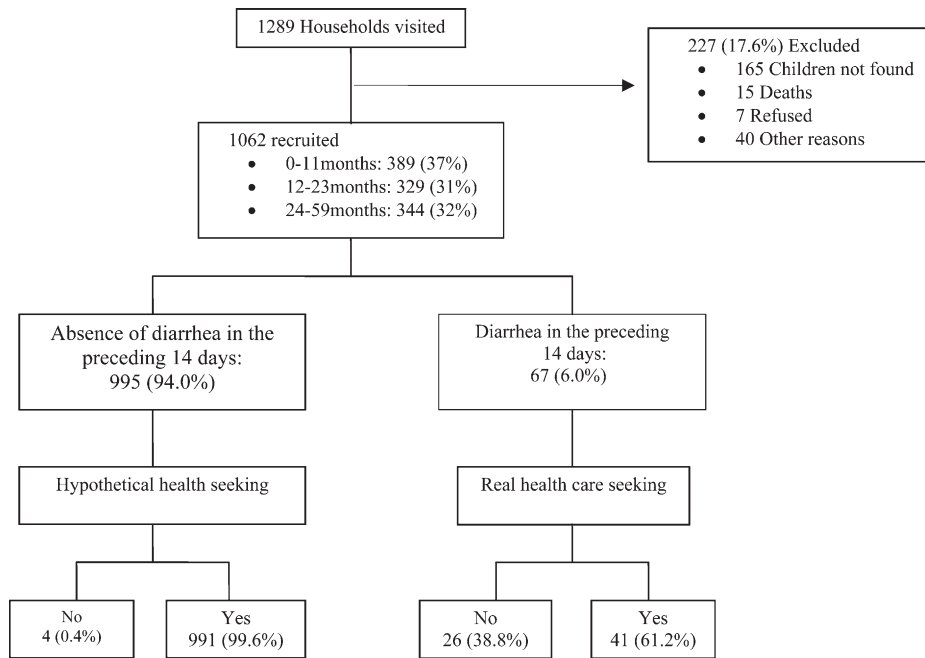


FIGURE 2. Study profile. Diarrhea and its relation to health services use among Manhica children (1^o survey).

and 79.2% (95% CI = 46.1–112.2) for children ages 24–59 months. Impression of parents or primary caretakers that their children did not seem to need care was the main cause of not seeking care outside home.

According to the multivariate model, the following variables were independently associated with use of health services: the presence of diarrhea with fever (OR = 4.69, 95% CI = 1.25–17.52, *P* = 0.022) and not knowing any sign of dehydration (OR = 15.08, 95% CI = 1.56–145.43, *P* = 0.020). Contrarily, having a television at home (OR = 0.21, 95% CI = 0.05–0.84, *P* = 0.029) was independently associated with a decreased use of the health facilities. There was no association between consultations at a healthcare structure and the level of education of the caretaker, distance to that health structure, adequate knowledge by the caretakers of the manifestations that define severe diarrheal disease, or adequate understanding of the necessary preventive measures against diarrhea. Other sources of care-seeking behavior were not significantly associated with any diarrheal disease (data not shown).

Treatment and fluid administration. Almost one-half of the children (29; 43%) did not receive any treatment at home before seeking care at the health center. However, among those caretakers administering treatments before going to hospital, herbal medication (30%) and oral rehydration salt (27%) were the most frequent referred treatments. When we enquired about “how much was offered to the child to drink during the diarrhea illness,” it was found that 11 (12%) of the mothers reduced or stopped their child’s usual liquid/breast milk intake, 47 (73%) of the mothers maintained the usual amount of liquid or breast milk, and only 9 (16%) of the mothers gave an increased amount of liquid or breast milk to their children with diarrhea. Although not significant (*P* = 0.082), these differences were more markedly noticeable in the youngest age group (infants), which were offered fewer liquids in relation to the older age groups. Only seven (12%) children with diarrhea were hospitalized. Among all patients

who sought care in health services on account of their diarrheal episode, 32 (48%) received oral rehydration solution, 13 (14%) received antibiotics, 8 (9%) received intravenous rehydration, and 16 (26%) received other different treatments.

Hypothetical health-seeking behavior. All primary caretakers whose child did not have diarrhea during the preceding 2 weeks were asked about their likely use of health centers should their children hypothetically develop diarrhea, and almost all respondents (991/995; 99%) stated that they would seek medical attention for any diarrheal episode. Commonly referred sources of healthcare seeking outside the household in that group were the health center/hospital (75–99%) or the traditional healer (0.1–13%). Relatively few children would be taken to the pharmacy (0.4–9.0%) or a friend (0.1–1%). The answers were similar, regardless of whether the putative diarrhea was with or without blood (data not shown).

Second survey. A total of 2,854 households was included in the second survey (Table 1); 880 children ages 0–11 months, 973 children ages 12–23 months, and 1,001 children ages 24–59 months represented 21%, 20%, and 59% of the DSS population, respectively. The male/female ratio of children included in the survey was 1. Of 2,854 children selected for inclusion, 246 caregivers (representing 7% of the DSS population) reported at least one episode of diarrhea during the recall period of 2 weeks. Of these 246 episodes, 103 (42%) episodes were considered MSD. The mean duration of a diarrhea episode was 4 days, and 213 (85%) of the children had a maximum number of three to six loose stools per day. The most common reported symptoms during the diarrhea episode were rice watery stool (44%), fever (42%), intense thirst (36%), vomiting (32%), and sunken eyes (30%). The risk of diarrhea decreased with increasing age (12–23 versus 0–11 months, OR = 0.82, 95% CI = 0.62–1.09; 24–59 versus 12–23 months, OR = 0.28, 95% CI = 0.19–0.42; *P* < 0.0001).

Of those caretakers reporting diarrhea during the recall period, 21 (41.5%, 95% CI = 25.9–57.1) in the first round,

TABLE 2

Factors independently associated with the use of health services in a multivariate adjusted analysis

Variables	Multivariate analysis			
	OR	95% CI		P value
		Lower	Upper	
1° Survey				
Diarrhea with fever	4.69	1.25	17.52	0.022
Not knowing any sign of dehydration	15.08	1.56	145.43	0.020
Having television at home	0.21	0.05	0.84	0.029
2° Survey				
Diarrhea with fever	1.88	1.01	3.51	0.046
Diarrhea with vomiting	2.78	1.53	5.08	< 0.001

36 (44.7%, 95% CI = 32.1–57.2) in the second round, 11 (43.6%, 95% CI = 22.1–65.1) in the third round, and 41 (44.0%, 95% CI = 32.9–55.1) in the fourth round used the health structures. Healthcare use increased in case of MSD to 51.8% (95% CI = 25.9–77.7) in the first round, 59.0% (95% CI = 41.6–76.5) in the second round, 70.6% (95% CI = 25.2–116.0) in the third round, and 54.2% (95% CI = 35.5–73.0) in the fourth round. Overall, healthcare use was 43.8% (95% CI = 36.9–50.6) and 56.9% (95% CI = 46.2–67.7) for total diarrhea and MSD, respectively. About 25% of the diarrhea cases sought care in the first day of diarrheal illness. Only two (1%) children with diarrhea were hospitalized. Impression of parents or primary caretakers that their children did not seem to need care was again the main cause of not seeking care outside home. Other sources of care seeking outside the home included seeing a traditional healer (6; 4%), directly buying medicines at shop/market (3; 3%), going to the pharmacy (3; 2%), and seeing an unlicensed practitioners (2; 2%).

Table 2 describes factors independently associated with seeking healthcare at a health center or hospital. The use of health services was significantly associated with diarrhea with fever (OR = 1.88, 95% CI = 1.01–3.51, $P = 0.046$) and vomiting (OR = 2.78, 95% CI = 1.53–5.08, $P < 0.001$). Once again, other sources of care seeking were not significantly associated with any diarrheal disease.

When enquired about “how much was offered to the child to drink during the diarrhea illness,” it was found that the majority (193; 79%) of the mothers reduced or stopped the child’s usual liquid/breast milk intake, whereas 3 (1%) caretakers maintained the usual amount of liquid or breast milk; only 49 (20%) caretakers provided an increased amount of liquid or breast milk to the children with diarrhea. Among all patients with a diarrheal episode, 136 (55%) received oral rehydration solution, 34 (16%) received home fluids, and 69 (28%) received no treatments. Within the preceding 14 days, the majority (185; 77%) of the children with diarrhea had improved, whereas some (6; 23%) continued with diarrhea.

DISCUSSION

The use of health services in case of illness is a complex behavior influenced by norms, moral values, beliefs, preferences, and socioeconomic potential as well as the perceived need of the users. Understanding the determinants of healthcare use in a determined population regarding specific illnesses may, therefore, provide useful information to improve their prognosis.

The study observed that, despite health access challenges in a rural area such as Manhiça, health services are used regularly from an early age by almost one-half (46–59%) of the children in their first year of life. However, these frequencies were lower than those numbers reported among children younger than 12 months of age (68.4%) in the general population of Mozambique in relation to the three most common infectious diseases together, namely malaria, diarrhea, and respiratory infections.²⁵

In the first survey, the final model of health services use was independently associated with diarrhea with fever, an easily recognized sign in a malaria-endemic region. This result is something expected and consistent with previous studies regarding the use of health services, which indicate that the main determinant of the use of services is the perceived need.^{26,27} However, high education, which has been described to be an important determinant of health service use,²⁸ was not shown to directly affect healthcare use when controlled by other cofounders. This result could be explained by the fact that, in Mozambique, the promotion campaigns of healthcare use are largely accomplished through the mass media (radio), and in Manhiça district particularly, healthcare use promotion is increased during DSS activities. Promotion campaigns consist of explaining to caretakers about problems that endanger life for the most prevalent diseases, such as malaria, acquired immunodeficiency syndrome (AIDS), and diarrhea, to serve as a warning. Thus, “not knowing any sign of dehydration,” a variable that may be associated with lower education, was found to be associated with increased healthcare use, possibly as a result of a lower capacity of the primary caretakers to perceive illness severity and monitor sick children, which may cause more severe episodes that require health center use. Contrarily, having television at home, a higher socioeconomic level variable, was associated with a marked decrease in the use of health services, suggesting that residents with high income have a tendency to use healthcare sources other than those sources provided by the national public system.

One of the main objectives of the second survey was to determine the proportion of care-seeking behavior from the DSS healthcare for MSD. Similar to the first survey, determinants of MSD were not associated to healthcare use. The use of health services was significantly associated with diarrhea with vomiting and fever. Vomiting was one of the most frequently reported symptoms by caretakers as a marker of disease severity in relation to the diarrheal episode, suggesting that it is widely used in the community as a red flag for diarrhea requiring urgent care.

In both the first and second surveys, the health structures were the main source of healthcare. Moreover, the other sources of care seeking were not significantly associated with any diarrhea, suggesting that the population considers DSS healthcare as the primary source of treatment of diarrhea. However, one must not minimize the misuse of over-the-counter medication and the potential role of traditional healers. Although their use was found to be low, the traditional healers are known to be the suppliers of herbal medication, which usually, is the first home treatment given to the child with diarrhea. The knowledge of the potential benefits or risks of some herbal medicines used in the community is still limited, and such treatments may even be detrimental to the diarrhea episode. This finding underlines the necessity for better communication between health professionals and caregivers regarding the use

of herbal therapy^{29,30} or any other medication without medical prescription. Future studies on the determinants of seeking care outside home and healthcare use among patients with diarrhea should investigate the above associations and local practices to further clarify the main determinants of healthcare use in rural communities such as Manhiça.

It is also remarkable that the vast majority of patients without episodes of diarrhea reported that they would use health services, possibly conditioned by the fact that the community associated the study interviewers with hospital staff. For this reason, the prevalence of the hypothetical use was almost 100%, thus precluding any analyses of risk factors associated with the hypothetical use. Although this result probably induced a significant response bias, it should be noted that the proportion of patients with MSD episodes who really used the health services was considerably high (86%) in any case.

The low prevalence of diarrhea found in this study (4.0–7.0% of patients enrolled) in a tropical country where diarrhea has been described as one of the major causes of pediatric morbidity and mortality is striking, but it is in line with other important decreasing trends for other important morbidity causes in the DSS area.^{12,16,31} However, it should be noted that, although each round of the second survey took 3–5 months and involved all DDS area, the first survey was restricted to a small study area, and additionally, it was performed between May and June, a cold and dry period, in which the prevalence and incidence of diarrhea in the area are lower than during the rest of the year. The results of diarrhea history characteristics are consistent with the literature.^{32,33}

In children, reduction of the usual diet intake is a common but not recommended practice during any diarrheal episode. The WHO recommends increasing the amount of liquid during diarrhea to avoid dehydration.^{34,35} Despite the considerable knowledge of the manifestations of dehydration and diarrhea severity by caretakers, according to our findings, liquid intake did not seem encouraged at the community level during a diarrheal episode, something that was even more markedly pronounced among infants than older children. Moreover, additional administration of oral rehydration salt was not commonly referred as the first home treatment of children with diarrhea in the first survey, but it was the first home treatment in the second survey. These fundamental perception mistakes regarding diarrhea management need to be urgently addressed. The promotion of breastfeeding and/or increased liquid intake during a diarrheal episode, plus the addition of oral rehydration salts as supporting medication, should become an essential part of any community-based training program to improve the prognosis of diarrheal disease.

It seems pertinent to make some methodological observations from the analysis of the strengths and limitations of this study that should be taken into account in the design of subsequent studies. The biggest strength was the continuous prospective monitoring realized in the second survey that revealed lower prevalence (43.75%) of healthcare use compared with the finding of the first cross-sectional study (65.16%); however, no important variation of healthcare use was seen during the continuous monitoring. Limitations of the study include the fact that a cross-sectional study design involves the measurement of cause and effect at the same time, introducing the problem of temporal ambiguity in establishing causal relationships, and a reporting bias related to the pres-

ence of the researcher, conditioned by the hypothetical relationship between the interviewers and paramedical staff. Finally, because of the characteristics of the sample in the first survey (small sample size in the group of children who had diarrhea and the small number of events in a category of the dependent variable hypothetical use), consideration of the hypothetical effect between variables (interactions) could not be analyzed; furthermore, it led to large 95% CIs that do not allow highly accurate estimates.

Limitations aside, it is possible to conclude that the use of national health services in case of diarrhea in children under 5 years is fundamentally associated with the perceived need; lower knowledge of dehydration signs and may be hampered by economic status. Community knowledge of the disease, its manifestations, and the risk factors associated with severity seemed adequate, contrary to the knowledge regarding best practices to treat such episodes, such as, for instance, the recommendation of increasing liquid intake. Understanding determinants of health services use may help to improve health planning. Additionally, the establishment of continuous prospective monitoring allows accounting for changes in healthcare use that may occur because of seasonality or secular events.

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**8.5 Article 5 : Severe malnutrition among children under the age of 5 years admitted
to a rural district hospital in southern Mozambique**

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Severe malnutrition among children under the age of 5 years admitted to a rural district hospital in southern Mozambique

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Abstract

Objective: To describe the burden, clinical characteristics and prognostic factors of severe malnutrition in children under the age of 5 years.

Design: Retrospective study of hospital-based data systematically collected from January 2001 to December 2010.

Setting: Rural Mozambican district hospital.

Subjects: All children aged <5 years admitted with severe malnutrition.

Results: During the 10-year long study surveillance, 274 813 children belonging to Manhica's Demographic Surveillance System were seen at out-patient clinics, almost half of whom (47%) presented with some indication of malnutrition and 6% (17 188/274 813) with severe malnutrition. Of these, only 15% (2522/17 188) were eventually admitted. Case fatality rate of severe malnutrition was 7% (162/2274). Bacteraemia, hypoglycaemia, oral candidiasis, prostration, oedema, pallor and acute diarrhoea were independently associated with an increased risk of in-hospital mortality, while malaria parasitaemia and breast-feeding were independently associated with a lower risk of a poor outcome. Overall minimum community-based incidence rate was 15 cases per 1000 child-years at risk and children aged 12–23 months had the highest incidence.

Conclusions: Severe malnutrition among admitted children in this Mozambican setting was common but frequently went undetected, despite being associated with a high risk of death. Measures to improve its recognition by clinicians responsible for the first evaluation of patients at the out-patient level are urgently needed so as to improve their likelihood of survival. Together with this, the rapid management of complications such as hypoglycaemia and concomitant co-infections such as bacteraemia, acute diarrhoea, oral candidiasis and HIV/AIDS may contribute to reverse the intolerable toll that malnutrition poses in the health of children in rural African settings.

Keywords
Malnutrition
Bacteraemia
Risk factors
Children
Mozambique

In developing countries, malnutrition, with the different spectrum of diseases that it comprises, is highly prevalent and contributes significantly to the premature death of children. Malnutrition is believed to play a key role in up to a third of the 8.8 million annual deaths occurring in children under the age of 5 years, and malnourished children have a fourfold increased risk of death⁽¹⁾. Significantly, sub-Saharan Africa carries the brunt of the impact caused by malnutrition, as almost half of its associated deaths occur there^(2,3).

Thus it appears that in order to decrease child mortality, one of the most relevant and pressing of the Millennium Development Goals, strategies to reduce both the prevalence

and consequences of malnutrition will need to be put in place⁽⁴⁾. Malnutrition is not a single disease, often underlies other conditions and comprises a wide spectrum of presentations that become evident principally as acute or chronic hindrances to the child's growth and development for a specific age. It arises from the combination of a series of intertwining environmental, nutritional, clinical, cultural and socio-economic determinants that need to be addressed in a comprehensive manner to reverse the vicious circle that leads to clinical disease. Clinical management of severely malnourished children is complex and challenging, requiring long hospitalization and a multidisciplinary approach. Updated WHO case management guidelines⁽⁵⁾ have been

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implemented in some African hospitals, but as a result of the existing weak and fragile health infrastructures, and the lack of specialized training to deal with such a complex condition, the management of malnutrition is often inadequate. Furthermore, the spread of the HIV pandemic in Africa and its impact on the nutritional status of sick children have triggered a secondary epidemic of severe malnutrition. The pathophysiology among severely sick malnourished children with AIDS and their clinical response to the accepted WHO therapeutic guidelines may be different from those of children with primary severe malnutrition secondary to food shortage rather than HIV infection.

As a strategy to improve case management of children hospitalized with severe malnutrition, the Manhiça Health Research Center (CISM), the Africa Viva Foundation and district health authorities started in 1999 a collaborative programme which includes continuous training of health workers to improve malnutrition diagnosis, management and post-discharge follow-up, assists with the provision of nutritional support through therapeutic foods and has enhanced the existing infrastructure by building a malnutrition-specific ward. In the current paper we present an analysis of the burden, clinical characteristics and risk factors for adverse prognosis associated with severe malnutrition among all patients <5 years of age admitted to Manhiça's District Hospital (MDH) from 2001 to 2010.

Materials and methods

Study site and population

The current retrospective study is based on information collected between January 2001 and December 2010 at MDH, the referral health facility for Manhiça district, a rural area in southern Mozambique with about 140 000 inhabitants. The area has been described in detail elsewhere⁽⁶⁾. The Centro de Investigação em Saúde de Manhiça (CISM) has been running a Demographic Surveillance System (DSS)⁽⁷⁾ in the area and a morbidity surveillance system at the MDH⁽⁸⁾. Almost half (49%) of the population lives beneath the poverty line of less than \$US 1 per day. Agricultural subsistence is the main activity in the district, together with work at the large sugar factories in neighbouring Maragra and Xinavane. Maize, sweet potatoes, peanuts and cowpea are the staple foods. Thirty-four per cent of the families are led by women (C Saco, personal communication, 2010). During the study period, other ongoing nutritional interventions at the community level organized by local agricultural government-based structures included the distribution and planting of sweet potato and the community supplementation of Plumpy'Nut and soyabean targeting HIV or tuberculosis patients, both adults and children. The area is endemic for malaria and the community prevalence of HIV is among the highest in the world, with prevalence rates reaching as high as 40% in the district⁽⁹⁾.

Severe malnutrition is the fourth commonest cause of hospital admissions.

Data collection and clinical management

MDH is a 110-bed hospital including a sixteen-bed specific malnutrition ward and admits about 4500 children per annum. Standardized forms are routinely completed for all out-patients and in-patients, and include demographic, clinical and laboratory data. Weight is measured for all out-patients but height is measured only for admitted patients. Mid upper-arm circumference is not routinely registered. Malaria is screened in all febrile patients. On admission, a single blood culture is performed for all children under the age of 2 years and for older children with a temperature $\geq 39^{\circ}\text{C}$ or with severe malnutrition or other signs of severe disease according to clinical judgement⁽¹⁰⁾.

Following national and WHO recommendations, empirical antimicrobial therapy is started on admission for children with severe malnutrition^(11,12), using as first line a parenteral combination of ampicillin plus gentamicin. Penicillin, cloramphenicol or ceftriaxone may alternatively be administered according to availability or clinical severity. Antibiotic therapy is re-assessed based on clinical response and blood culture results. According to standard recommendations, malnourished children also receive mebendazole and are supplemented with vitamin A, multivitamins and Fe (from week 2 onwards).

Nutritional supplementation with enriched milk formulas (F75 and F100), or with a hospital-made mixture of milk plus oil and sugar, is administered according to standard protocols. Weight is measured and re-assessed daily by a trained nurse using a hanging scale (infants) or an electronic floor scale (older ones) so as to assess the evolution and calculate nutritional requirements. Scales are calibrated routinely. Additional solid feeding is introduced as soon as possible according to age, appetite and resolution of oedemas. All complications and conditions associated with malnutrition, including hypothermia, hypoglycaemia, dehydration, sepsis, shock or heart failure, are assessed and managed according to standard WHO recommendations⁽⁵⁾. Immunization status is revised and updated at discharge for all children if necessary.

Clinical definitions

Weight-for-age (underweight), weight-for-height (wasting) and height-for-age (stunting) Z-scores were calculated for each child admitted during the study period using standard deviations and US growth charts⁽¹³⁻¹⁵⁾. We defined the types of malnutrition according to the different parameters calculated (weight-for-age, weight-for-height and height-for-age) and divided the study population into three malnutrition groups: severe (Z-score ≤ -3), mild to moderate (Z-score > -3 and < -1) or non-existent (Z-score > -1). Anaemia was classified as severe if packed cell volume was $< 25\%$ in neonates or $< 15\%$ for



other paediatric age groups or non-severe if between 25 and 42% in neonates or between 15 and 32% for older infants. Increased respiratory rate was defined according to age standard definitions⁽⁵⁾. Hypoglycaemia was defined as severe if glycaemia on admission was <2.2 mmol/l; or moderate if between 2.3 and 3.0 mmol/l. Deep coma required a Blantyre coma score ≤ 2 . Dehydration was defined according to standard WHO protocols⁽¹⁶⁾. Bacteraemia required the isolation of pathogenic non-contaminant bacteria in the blood.

Statistical analysis

Questionnaires were double entered in FoxPro-designed databases version 2.6 and statistical analyses performed with the STATA statistical software package version 9.0. Due to the characteristics of the sample (for 14–18% of the cases, we were unable to define height-for-age or weight-for-height status owing to missing height data), analytical inference was restricted to the weight-for-age indicator only. Recurrence of severe malnutrition was calculated considering a lag period of 30 d after each episode of severe malnutrition with a confirmed previous favourable discharge (thus excluding transferred or absconding children from the hospital). Minimum community-based incidence rates (MCBIR) were calculated by referring malnutrition cases to population denominators, establishing the time at risk (child-years at risk (CYAR)), inferred from the DSS information. Negative binomial regression models were estimated to compare incidence rates between age groups or calendar years. Models were estimated with a random intercept to take into account repeated measures, since children can belong to several age categories or to several calendar years during the follow-up. Overall P values for age and calendar year were calculated using the likelihood-ratio test. Person-time was excluded after the first episode of severe malnutrition. Case fatality rates (CFR) were calculated by considering children with a known outcome at discharge (death or discharged) and represent in-hospital mortality. Proportions were compared using the χ^2 test or Fisher's exact test and odds ratios and 95% confidence intervals were estimated using logistic regression. Wilcoxon rank-sum tests were used for non-parametric comparisons. A multivariate logistic regression analysis was performed to assess independent risk factors for death among severely malnourished cases, using an automated backward stepwise estimation. Given that 3% (72/2522) of the severely malnourished children admitted MDH were transferred to Maputo's Central Hospital, 7% (169/2522) absconded from hospital prior to a discharge decision by the caring physician and that the dependent variable was the final outcome (dead/alive), only children with a known outcome were included in the analysis. All variables that were associated with death at a significance level of $P < 0.10$ in the univariate analysis were included in the multivariate model. The significance level for

removal from the model was set at $P = 0.06$ and that for addition to the model at $P = 0.05$.

Results

During the 10-year long study period, 274 813 children from the DSS area aged <5 years visited at the out-patient department of MDH. Six per cent (17 844/274 813) were subsequently admitted to the hospital, 52% of whom were male.

Six per cent (17 188/274 813) of all out-patients could be classified according to their weight-for-age as severely malnourished, but only a small proportion (14.6%; 2522/17 188) ended up being admitted (Figure 1). Almost half of all visiting children (47%; 128 652/274 813) suffered from some degree of malnutrition (mild, moderate or severe). Table 1 presents the proportion of in-patients according to age group (older or younger than 24 months of age) classified according to type and degree of malnutrition.

Recurring admissions in malnourished children were frequent. Indeed, from the 2522 severe malnutrition episodes, 1576 children were admitted once, 281 children were admitted twice, sixty-two children were admitted three times and further thirty-eight children were admitted more than three times. Figure 2 presents absolute numbers of malnutrition cases and deaths according to calendar year of admission.

Clinical features of children admitted with severe malnutrition

The prevalence of severe malnutrition was 11% (626/5672) among hospitalized infants aged <12 months, 23% (1169/5158) for children aged 12–23 months, 14% (449/3169) for children aged 24–35 months and 10% (278/2844) for children 36–59 months of age ($P < 0.001$). Table 2 summarizes the clinical and demographic characteristics of the 2522 severely malnourished children <5 years of age admitted during the study period, and compares them with all other non-severely malnourished admissions to hospital in this same age group. Children admitted with severe malnutrition were significantly younger than other admissions (mean age 20 *v.* 21 months, respectively; $P = 0.007$). Admissions with severe malnutrition seemed to occur more frequently during the rainy season, the busiest period for the hospital, similarly to what occurred with the rest of admissions ($P = 0.008$). Children with severe malnutrition also appeared more significantly ill, and reported symptoms and witnessed signs were generally more frequent among severely malnourished children than in those better nourished. With the exception of malaria parasitaemia, highly prevalent among severely malnourished patients (52%) but significantly more frequent among non-severely malnourished patients (65%, $P < 0.001$), coexisting morbidities (pneumonia, acute diarrhoea, severe anaemia or

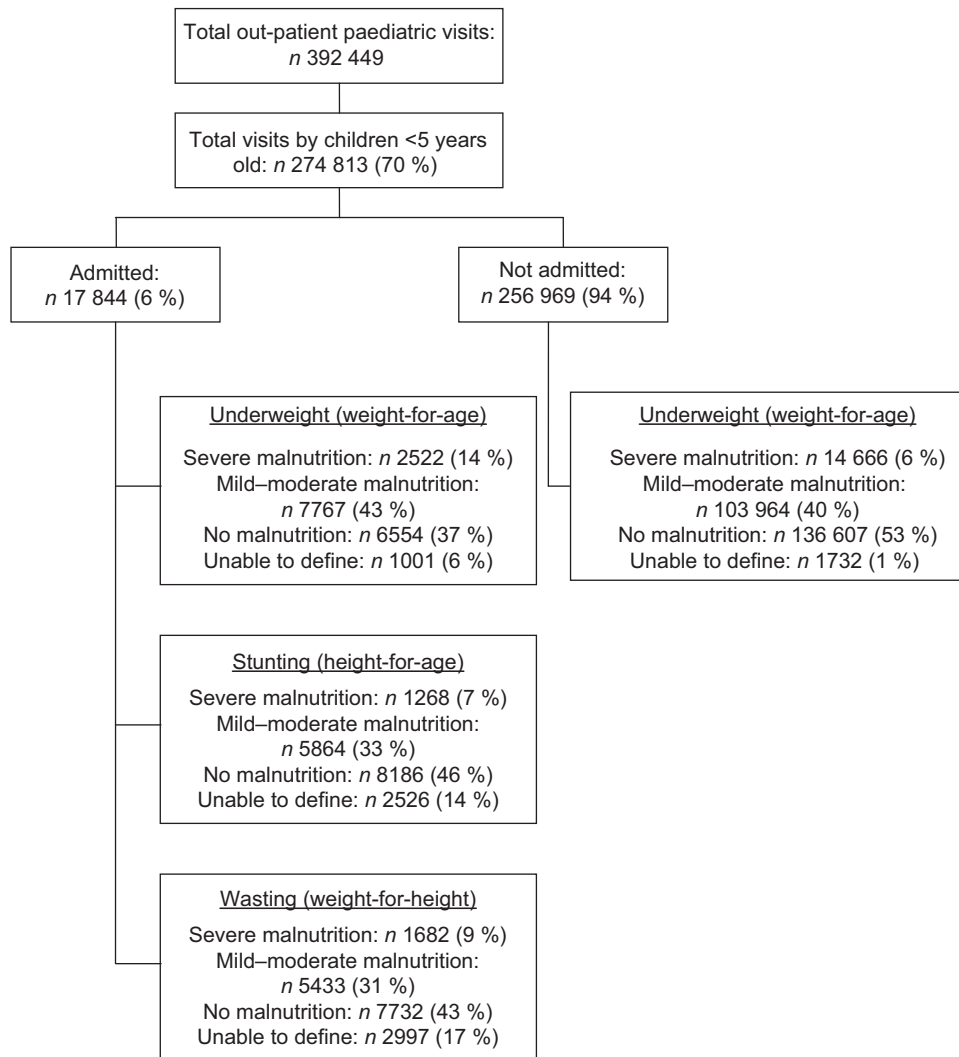


Fig. 1 Distribution of malnutrition among southern Mozambican children aged <5 years covered by Manhica's Demographic Surveillance System, who visited as out-patients or were admitted to Manhica District Hospital (from January 2001 to December 2010)

Table 1 Age distribution of malnutrition among southern Mozambican children aged <5 years covered by Manhica's Demographic Surveillance System, who were admitted to Manhica District Hospital (from January 2001 to December 2010)

Z-score	Age <24 months		Age 24–59 months		P
	n/N	%	n/N	%	
Weight-for-age					
>−1	4007/10 830	37	2547/6013	42	
<−1 and >−3	5028/10 830	46	2739/6013	46	
≤−3	1795/10 830	17	727/6013	12	<0.001
Height-for-age					
>−1	5824/10 028	58	2362/5290	45	
<−1 and >−3	3534/10 028	35	2330/5290	44	
≤−3	670/10 028	7	598/5290	11	<0.001
Weight-for-height					
>−1	4679/9561	49	3053/5286	58	
<−1 and >−3	3587/9561	37	1846/5286	35	
≤−3	1295/9561	14	387/5286	7	<0.001

hypoglycaemia) were all significantly more frequent in the severe malnutrition group. Median duration of hospitalization in children was also significantly prolonged

in severely malnourished patients (7 (IQR 3–9) d *v.* 3 (IQR 2–5) d, $P < 0.001$). CFR were significantly higher ($P < 0.001$) for patients with severe malnutrition

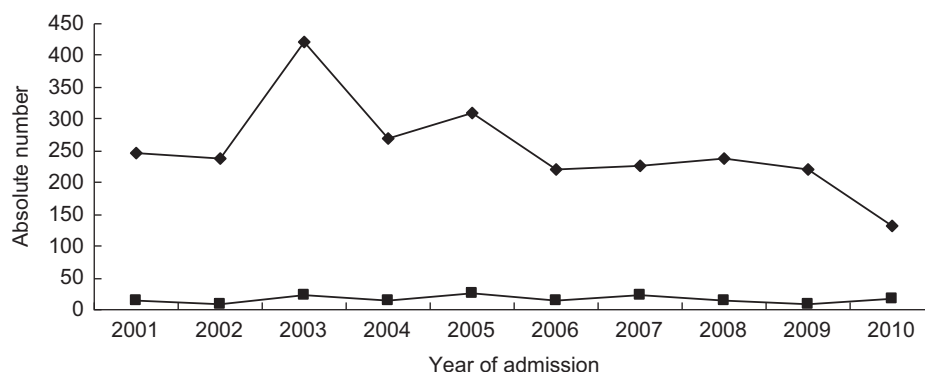


Fig. 2 Absolute numbers of annual admissions with severe malnutrition (—◆—) and related deaths (—■—) among southern Mozambican children aged <5 years, Manhiça District Hospital (from January 2001 to December 2010)

Table 2 Demographic and clinical characteristics of southern Mozambican children aged <5 years admitted to Manhiça District Hospital with severe malnutrition (from January 2001 to December 2010), compared with all other non-severely malnourished admissions in the same age group (mild and moderate malnutrition and non-malnourished)

	Severe malnutrition (n 2522)		Other admissions (n 14 321)		P
	n	%	n	%	
Demographic characteristics					
Sex					
Male	1365	54	7920	55	0.272
Age (months)					
Mean	20		21		
SD	11		15		0.007
Season					
Rainy	1573	62	8528	60	0.008
Breast-feeding*	803	61	4825	79	<0.001
Symptoms					
Cough	1836	73	9724	68	<0.001
Reported breathing problems	608	24	3104	22	0.006
Vomiting	692	28	3355	23	<0.001
Convulsions	107	4	1263	9	<0.001
Stop eating	298	13	1212	9	<0.001
Stop drinking or suckling	199	8	842	6	<0.001
Signs					
Temperature (°C)					
Mean	37.7		38.0		
SD	1.3		1.3		<0.001
Pallor (mucosal)	518	21	2350	16	<0.001
Dehydration	647	26	2174	15	<0.001
Oedema	298	12	524	4	<0.001
Ear discharge	110	4	374	3	<0.001
Liver palpable	101	4	392	3	0.001
Spleen palpable	593	24	3768	26	0.003
Prostration	281	16	1231	14	0.021
Deep coma (Blantyre coma score ≤2)	123	5	578	4	0.052
Co-morbidities					
Malaria parasitaemia	1258	52	9080	65	<0.001
Pneumonia	681	27	3282	23	<0.001
Bacteraemia	261	12	785	7	<0.001
Acute diarrhoea	784	31	2959	21	<0.001
Severe anaemia	312	12	1390	10	<0.001
Hypoglycaemia	133	6	534	4	0.034
Oral candidiasis	155	6	248	2	<0.001
Outcomes					
Admission length (d), mean and IQR	7	3–9	4	2–5	<0.001
Case fatality rate, n/N and %†	162/2274	7.1	222/13 619	1.6	<0.001

IQR, interquartile range.

*Denominators only include children aged <24 months.

†Denominators are different from total admitted patients in each group as they only include children with known outcome (absconded and transferred children excluded).



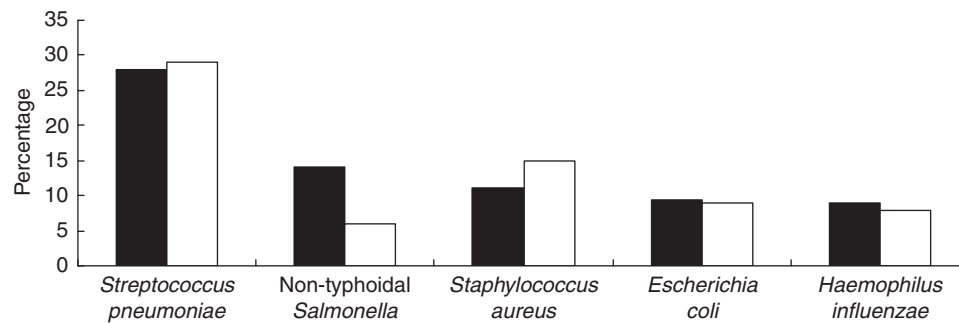


Fig. 3 Relative contributions of the main five bacterial isolates as causes of bacteraemia among severely malnourished (■) southern Mozambican children aged <5 years, compared with all other non-severe malnutrition diagnoses (□), Manhica District Hospital (from January 2001 to December 2010)

(162/2274; 7.1%) when compared with all other non-severely malnourished children (222/13 619; 1.6%) and importantly the risk of death seemed to increase in parallel with the degree of malnutrition (no malnutrition *v.* mild: OR = 1.16, 95% CI 0.83, 1.61; mild *v.* moderate malnutrition: OR = 1.76, 95% CI 1.26, 2.46; moderate *v.* severe malnutrition: OR = 2.76, 95% CI 2.09, 3.63; $P < 0.001$).

The most common accompanying clinical diagnoses in children with severe malnutrition, as indicated by the discharging clinician, were malaria (52%), acute diarrhoea (31%), pneumonia (27%), bacteraemia (12%) and severe anaemia (12%). Bacteraemia was significantly more common among children with severe malnutrition than among children without it (12% *v.* 7% in all other admissions; $P < 0.001$). Figure 3 shows the aetiology of bacteraemia in patients with severe malnutrition, as compared with the aetiology of bacteraemia for all other admissions. The most prevalent pathogens isolated from blood culture in malnourished children were *Streptococcus pneumoniae* (28%), non-typhoidal *Salmonella* (14%), *Staphylococcus aureus* (11%), *Escherichia coli* (9%) and *Haemophilus influenzae* (9%). *S. pneumoniae* (28% *v.* 29%, $P < 0.001$) and *S. aureus* (11% *v.* 15%, $P < 0.001$) were the only pathogens significantly less frequently isolated among severely malnourished children.

Risk factors for poor outcome among admitted children with severe malnutrition

Of the 162 deaths among severely malnourished children occurring during the study, 78% (126/162) occurred in children <24 months of age. CFR for severely malnourished children were significantly higher in the younger age groups: 12% (65/545) for infants, 6% (61/1068) for children aged 12–23 months and 5% (36/661; $P < 0.001$) for those aged >24 months. Independent risk factors for death among severely malnourished patients according to age group are shown in Table 3. Importantly, oral candidiasis and prostration independently increased the odds of death while malaria parasitaemia (both age groups) and breast-feeding (for those aged <24 months only)

Table 3 Independent risk factors for severe malnutrition death by multivariate analysis among southern Mozambican children aged <5 years, Manhica District Hospital (from January 2001 to December 2010)

Variable	Multivariate analysis			P
	OR	95% CI		
		Lower	Upper	
Children aged <24 months				
Breast-feeding	0.57	0.33	0.99	0.045
Prostration	3.20	1.77	5.80	<0.001
Oral candidiasis	3.32	1.70	6.48	<0.001
Hypoglycaemia	2.87	1.21	6.78	0.016
Bacteraemia	1.95	1.00	3.78	0.049
Malaria parasitaemia	0.49	0.26	0.95	0.034
Children aged 24–59 months				
Pallor	3.35	1.05	10.63	0.041
Oral candidiasis	20.15	2.87	141.62	0.003
Oedema	10.77	2.49	46.70	0.001
Acute diarrhoea	4.39	1.49	12.91	0.007
Prostration	17.38	4.36	69.20	<0.001
Malaria parasitaemia	0.30	0.09	0.92	0.035

were independently associated with a lower risk of a poor outcome.

Minimum community-based incidence rates

Table 4 show the age-specific MCBIR for severe malnutrition in the study area. Overall incidence was 15 cases per 1000 CYAR. The number of cases does not coincide with the numbers presented above, as MCBIR were calculated only for the first episode of severe malnutrition per child. During the study period, the incidence of severe malnutrition declined significantly from 33/1000 CYAR in 2001 to 7/1000 CYAR in 2010 ($P < 0.001$). The decline was higher in the period from 2001 to 2002 and mostly observed in the age groups 12–23 months and 24–59 months, in which it dropped by almost 50%. The risk of severe malnutrition increased rapidly with age up to 24 months (OR = 2.23; 95% CI 1.94, 2.55), but then subsequently decreased (OR = 0.48; 95% CI 0.40, 0.57). MCBIR for severe malnutrition for the whole study period was 20/1000 CYAR in children aged 0–11 months

Table 4 Minimum community-based incidence rate (MCBIR) of admitted severe malnutrition cases per 1000 child-years at risk (CYAR), by year of study, among southern Mozambican children aged <5 years, Manhiça District Hospital (from January 2001 to December 2010)

Calendar year	Population	Cases	Time at risk (CYAR)	MCBIR (cases per 1000 CYAR)	IRR	95 % CI	<i>P</i>
2001	8525	212	6453.1	32.85	1.00	–	<0.0001
2002	15 139	171	9115.6	18.76	0.62	0.50, 0.77	
2003	16 520	298	12 329	24.17	0.92	0.75, 1.12	
2004	16 474	199	12 514	15.90	0.67	0.54, 0.84	
2005	18 269	222	13 720	16.18	0.67	0.53, 0.83	
2006	18 631	182	14 164	12.85	0.52	0.41, 0.65	
2007	19 005	190	14 493	13.11	0.50	0.40, 0.63	
2008	19 364	189	14 792	12.78	0.49	0.39, 0.61	
2009	19 412	183	14 867	12.31	0.46	0.36, 0.58	
2010	19 710	112	15 061	7.44	0.27	0.21, 0.35	
TOTAL	51 778	1958	127 507	15.36			

IRR, incidence rate ratio.

P from negative binomial regression model with random effects using the likelihood-ratio test.

NB: convergence was not achieved; estimates are based on iterated maximization.

and 35/1000 CYAR in children aged 12–23 months. Children from 24 to 59 months of age had the lowest MCBIR (7/1000 CYAR; $P < 0.001$).

Discussion

The present study aimed to investigate the burden, clinical characteristics and risk factors associated with death among out-patients and hospitalized Mozambican children with severe malnutrition. Surprisingly, the majority (>75%) of those children seen as out-patients and classified as severely malnourished according to their weight-for-age *Z*-score were not admitted to hospital. As current recommendations suggest that all severely malnourished children should be admitted, these findings indicate a massive failure to correctly identify paediatric malnutrition at the first encounter of the patients with the health facility^(17,18). In rural areas of sub-Saharan Africa, this first contact usually relies on health personnel with limited medical training and is a critical moment for the correct recognition of a common condition that entails an unacceptably high CFR (>7% in our series). This very limited recognition and admission of severely malnourished children represents a missed opportunity to identify children who could benefit significantly from measures to increase their likelihood of survival, and calls for an immediate need for training of health personnel in the screening and identification of signs and symptoms associated with severe malnutrition. Even though many of these patients may have consulted with banal diseases, lack of recognition surely did prevent them from receiving appropriate management and nutritional advice to improve their derisory nutritional status.

The high prevalence (6–14%), high associated CFR (7%), clinical features and seasonal pattern of severe malnutrition found in the present study seem in general agreement with those described for other neighbouring areas^(10,19–22). CFR among hospitalized children increase

with increasing severity of malnutrition, peaking at about 7% for those children in the lowest malnutrition category (*Z*-score ≤ -3), and confirm the high morbidity and mortality burden associated with severe malnutrition in children under 5 years in the region and the massive underlying role that malnutrition plays among admitted patients, potentially impacting all-cause diagnoses on admission. Furthermore, it is also remarkable that 40–43% of the children presented mild-to-moderate malnutrition. These results indicate a need for future efforts in identifying these children at risk of developing severe malnutrition at the first stages in order to provide adequate and timely preventive and therapeutic interventions.

Multivariate analysis showed that the presence of oedema, prostration and hypoglycaemia was each independently associated with an increased risk of death among severely malnourished children, suggesting that efforts should be made for an early screening of these complications at the health facility. While the diagnosis of oedema or prostration implies the recognition of simple clinical signs by health workers, identifying hypoglycaemia is clinically challenging as it depends on the availability of more costly devices. Pallor, a severe anaemia sign, was also identified as an independent risk factor for death in children with severe malnutrition. Screening and prevention of anaemia should therefore be performed among all malnourished children when attending the out-patient services in rural areas, especially in malaria-endemic countries where this infection may also contribute significantly to anaemia. Although conflicting evidence surrounds the routine administration of Fe supplements for the prevention of anaemia in malaria-endemic areas and current recommendations suggest withholding Fe for at least 1 week after malnutrition treatment has been initiated⁽²³⁾, anaemia remains one of the major risk factors for a poor outcome among severely malnourished children and measures to prevent and treat it should be a priority.

The preventive impact of breast-feeding on malnutrition is significant^(24,25) and is well established, similarly to



what we have shown in our series, particularly when breast-feeding is exclusive in the first 6 months of life and continued with safe, appropriate and adequate complementary feeding up to 2 years of age or even beyond^(26,27). Taking into account that data regarding the kind of breast-feeding (exclusive or mixed) and associated individual hygienic, socio-economic and sanitation conditions (all factors with an important impact on the risk of malnutrition) were not adequately collected, future studies should explore the effect of the above-mentioned variables on the health of Manhiça district's children.

Data on HIV/AIDS co-infection, already highly prevalent in Manhiça district at the time, were unfortunately not routinely collected during the period covered by the present retrospective study, and HIV serostatus is unknown for these patients. However, it is likely that HIV infection – suspected by typical signs such as the presence of oral candidiasis – would have emerged as an important factor contributing to the development of malnutrition and, once established, as a prominent independent risk factor for death^(28,29). Thus, measures to prevent mother-to-child transmission of HIV may very well be critical to decrease the impact that this infection imposes in the nutritional status of children and the prognosis of malnourished children. Moreover, the concomitant presence of bacteraemia and acute diarrhoea was confirmed in our study as other independent risk factors for death among malnourished patients. Malnutrition *per se* is a well-established risk factor for infections^(10,30). This frequent complication, independently associated with a poor prognosis, supports the obligate addition of wide-spectrum antibiotic coverage in any patient admitted with a diagnosis of severe malnutrition, so as to cover the most frequent bacteria found in this specific group of patients which, as other studies have worryingly shown, are becoming increasingly resistant to first-line therapies⁽³¹⁾.

Conversely, the presence of malaria parasitaemia was shown in our series to be independently associated with a decreased risk of dying. The supposed protection conferred by malaria parasites among malnourished children is a highly controversial issue, the pathophysiological bases of which are difficult to understand. While previous studies have shown either no significant association between the two diseases^(32–34) or even an increased risk of malaria morbidity among malnourished children^(35,36), a controversial study from Papua New Guinea suggested that malnutrition may protect children from malaria⁽³⁷⁾. Methodological differences related to malnutrition definitions and in the age of children included were proposed to explain the discrepant results between the aforementioned studies. In the current study, those children presenting with malaria parasitaemia may have had a lower CFR because the direct cause of admission (i.e. the malaria infection) responds rapidly to treatment, possibly causing an early discharge motivated by resolution of the malaria episode but without taking into

account the outcome of the associated and possibly unresolved malnutrition episode.

We also found that the risk of severe malnutrition increased rapidly until 24 months of age and then subsequently decreased. Risk of death, however, decreased uniformly with increasing age. The type of nutrient requirements and the physiological processes in less mature children (<24 months) compared with older children may explain this pattern. Moreover, the former group is also more heterogeneous in terms of underlying aetiologies and pathophysiology than older children.

Clinical management of severely malnourished children requires a multidisciplinary approach and long hospitalization that includes an initial period beginning on admission to hospital and lasting until the child's condition has stabilized, usually after a minimum of 7 d. Follow-up and post-discharge monitoring are also part of the malnutrition management and are critical to prevent recurrences, common in this spectrum of diseases. However, and most importantly, adequate preventive measures need to be put in place to guarantee that the vicious circle leading to malnutrition does not occur in the first place. Judging by the high burden of malnutrition reported in the present study, it is clear that the few ongoing governmental-organized preventive activities at the community level are insufficient. Moreover, although CISM and the Manhiça district health authorities have an intra-hospital long-standing collaboration that started in 1999 with a clear aim to improve the detection and management of admitted malnourished patients, our findings reveal important functioning failures and important limitations of the programme. First, although the median duration of hospitalization in children with severe malnutrition was 7 d, a sufficiently long period for an appropriate initial phase treatment, an important proportion of the severely malnourished cases (7%, 169/2522) absconded from hospital prior to a discharge decision by the caring physician, possibly contributing to the highly frequent re-admissions. Furthermore, with the exception of an initial and final decrease of its incidence (as measured by MCBIR, calculated only for the first episode) coinciding with the first and tenth years of the programme's activities, no subsequent significant variations in malnutrition incidence rates could be observed in the intervening years of programme activities. Additionally, absolute numbers of severe malnutrition cases and related deaths remained similar during the programme's implementation period. As previously mentioned, the rampant HIV/AIDS pandemic, highly prevalent at the community level and with a clearer higher impact after year 2005, may have significantly worsened the clinical evolution and prognosis of these patients^(6,38). Altogether, these findings support the theory that malnutrition is a complex medical emergency requiring a multidisciplinary approach. Improvements in its incidence rely not only on the availability of a well-functioning and accessible public



health system, but also on parallel enhancements in the community's socio-economic status. More extensive measures to combat poverty and hunger and promote social inclusion, as well as a wider application of community education targeting the prevention of malnutrition, are aspects to be urgently considered by national public health nutrition policy makers.

The present study has other methodological limitations worth mentioning, including the fact that it retrospectively looks at a 10-year long series of patients, a fact limiting the interpretation of results and the direction of causality associations. Prospective studies taking into account the nutritional status of children in the hospital are therefore suggested. Finally, although height should be measured at admission, 14–18% of the children had an incorrect registration of this indicator. The unavailability of height and other anthropometric data for all patients made an accurate assessment of malnutrition difficult. However, weight-for-age remains a well-established and accepted methodology for evaluating nutritional status because of the difficulties related to the monitoring of other anthropometric data in routine clinical practice. Nevertheless, the development of strategies to educate and qualify professionals working in hospitals in order to enhance the value of current techniques to assess nutritional status in patients attending hospitals, focusing on the paediatric population, should be reinforced.

Conclusion

Severe malnutrition among admitted children in this area of southern Mozambique is common but frequently undetected, despite its associated high risk of death. Measures to improve its recognition by clinicians responsible for the first evaluation of patients at the outpatient level are urgently needed, so as to improve their likelihood of survival. Together with this, the rapid management of complications such as hypoglycaemia and concomitant co-infections such as bacteraemia, acute diarrhoea and HIV/AIDS may contribute to reversing the intolerable toll that malnutrition poses in the health of children in rural African settings.

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9. SUMMARY OF RESULTS AND CONCLUSIONS

9.1 Article 1

Burden and etiology of diarrheal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study

Results

Moderate-to-severe diarrhea

- During the 36-month case-control study period, 14,753 children met enrolment criteria for moderate-to-severe diarrhea, of these 9439 were included in the analysis and concomitantly, 13,129 matched controls were enrolled.
- Sixty days after enrolment, follow-up was performed for 8549 (91%) of patients with moderate-to-severe diarrhea known to be alive at discharge from the DSS health centers and 12,390 (94%) of enrolled control children.
- One or more putative pathogens were identified in 7851 (83%) children with moderate-to-severe diarrhea and in 9395 (72%) controls; two or more agents in 4200 (45%) cases and 4075 (31%) controls.
- The median proportion of episodes attributable to a pathogen was 44% (IQR 41–52) for infants, 47% (21–52) for toddlers, and 40% (23–53) for children.
- Four pathogens were significantly associated with moderate-to-severe diarrhea at all seven study sites in one or more age strata: rotavirus, *Cryptosporidium*, *Shigella*, and ST-EPEC (ST-only or LT/ST strains).
- Rotavirus had the highest AF of any pathogen at every site during infancy, and although its AF generally diminished with age, rotavirus had the largest AF of any pathogen in toddlers at four sites, and at the Mali and India sites even among the eldest stratum.

- *Cryptosporidium* had the second highest AF during infancy at five sites, persisting in importance, albeit at a lower level, during the second year of life at five sites; in the eldest stratum.
- The adjusted AF of *Shigella* increased from infants to toddlers at every site, rising to the rank of first or second in AF at four sites in toddlers and five sites in the eldest stratum.
- ST-EPEC was a significant pathogen at every site in at least one age stratum and in all age strata at four sites.
- A small proportion (<5%) of moderate-to-severe diarrhea was attributable to adenovirus 40/41 at six sites during infancy, and in three sites during the second year of life.
- Three enteropathogens showed regional importance. *Aeromonas* was a leading pathogen in the Pakistan and Bangladesh sites, with the peak AF at age 24–59 months. *V cholerae* O1 appeared in an age-escalating pattern in the three Asian sites plus Mozambique. *C jejuni* was significantly associated with moderate-to-severe diarrhea in at least one age stratum at the three Asian sites.
- *Giardia* was significantly negatively associated with moderate-to-severe diarrhea; and in univariate analyses *Giardia* was identified significantly more frequently in controls than in patients with moderate-to-severe diarrhea aged 12–59 months in ten of the 14 age-site strata.
- DSS-wide annual incidence rates of moderate-to-severe diarrhea at all sites combined due to rotavirus dominated during the first 2 years of life, and during the infancy the incidence (7.0 episodes per 100 child-years, 95% CI 5.4–8.5) was more than double that of any other pathogen.

- Regardless of the age stratum, the estimated incidence of moderate-to-severe diarrhea was highest in India, next highest in Kenya and Mali, and lowest in The Gambia, Pakistan, Bangladesh, and Mozambique.
- The overall annual incidence of moderate-to-severe diarrhea per 100 child-years was 30.8 (95% CI 24.8–36.8) for infants, 23.1 (95% CI 17.2–29.0) for toddlers, and 7.7 (95% CI 3.9–11.5) for children.

Malnutrition

- Mean height-for-age z-score at enrolment in patients with moderate-to-severe diarrhea and controls was considerably below the WHO reference for infants and, with one exception, deviated further from the reference at older ages however linear growth faltering was especially marked at the Pakistan site.
- Height-for-age z-score (HAZ) of moderate-to-severe diarrhea cases decreased between enrolment and follow-up (ie, negative δ HAZ), with only one exception (Malian children aged 24–59 months); the decline was significantly greater in patients with moderate-to-severe diarrhea than in controls in most site-age strata and in all age strata in the pooled analysis, after adjusting for enrolment height-for-age z-score and time to follow-up.

Mortality

- During follow-up within 90 days of enrolment, 190 (2.0%) deaths were detected in the 9439 children enrolled with moderate-to-severe diarrhea, and 37 (0.3%) deaths were detected in the 13 129 control children (OR 8.5, 95% CI 5.8–12.5, $p < 0.0001$).
- Mortality in children with moderate-to-severe diarrhea was highest in the Mozambique site, followed by The Gambia and Kenya, Pakistan and Mali, and finally Bangladesh and India.

- Mortality in patients with moderate-to-severe diarrhea exceeded mortality in controls at all sites and the differences were significant everywhere except in India.
- In patients with moderate-to-severe diarrhea, 64 (34%) of deaths occurred on days 0-7 after enrolment, 63 (33%) on days 8-21, and 63 (33%) after day 21; controls survived significantly longer than did patients with moderate-to-severe diarrhea ($p < 0.0001$ by logrank test).
- 105 (55%) of all deaths occurred at home or outside of a medical facility.
- Most deaths in patients with moderate-to-severe diarrhea occurred in infants (107 [56%]) and toddlers (60 [32%]). Even so, the weighted risk of mortality remained high in the oldest stratum in The Gambia (1.8%), Kenya (2.3%), and Mozambique (3.9%).
- In multiple Cox regression analysis, pathogens associated with a higher risk of dying in patients with moderate-to-severe diarrhea were ST-EPEC and typical EPEC in infants and *Cryptosporidium* in toddlers.
- By adjusting for site, enrolment HAZ was inversely associated with risk of dying in patients with moderate-to-severe diarrhea in all age groups, as follows: 0-11 months HR 0.62 (95% CI 0.54-0.72, $p < 0.0001$); 12-23 months HR 0.74 (95% CI 0.63-0.87, $p = 0.0002$); and 24-59 months HR 0.47 (95% CI 0.38-0.57, $p < 0.0001$).

Conclusions

- Moderate-to-severe diarrhea is common in the pediatric populations studied, producing more than 20 episodes per 100 child-years during each of the first 2 years of life.

- Rotavirus, *Cryptosporidium*, STETEC, *Shigella*, and, to a lesser extent, adenovirus 40/41 were the pathogens that contributed most attributable moderate-to-severe diarrhea cases.
- Mean height-for-age z-score at enrolment in patients with moderate-to-severe diarrhea and controls was considerably below the WHO reference, however children with moderate-to-severe diarrhea experienced a substantial nutritional insult, evidenced by significantly more linear growth faltering during the follow-up period compared with their matched controls.
- The risk of dying in patients with moderate-to-severe diarrhea was greatest in the sites included for the study. Most deaths occurred outside health facilities and were detected only because the study included a follow-up home visit about 60 days after enrolment.
- The substantial burden of moderate-to-severe diarrhea in sub-Saharan Africa and south Asia and its close association to malnutrition show that preventive strategies targeting pathogens independently associated with MSD could potentially reduce this disease and its sequelae by about 40% during the first 2 years of life.
- An urgent need exists to accelerate introduction or improve implementation of existing interventions with proven effectiveness, such as rotavirus vaccination and adjunct treatment of acute diarrhea with zinc and to revitalize diarrheal disease case management algorithms shown to reduce malnutrition.

9.2 Article 2

Diarrheal disease in rural Mozambique. Part I: Burden and etiology of diarrheal disease among children aged 0-59 months

Results

Burden of disease

- Over the period 2001-2012, all age groups showed a steady decline in the incidence of acute diarrhea that represents an 88% drop in the older age group, a 77% in the 12-23 months and a 76% in the youngest group.
- Estimations of weighted annual incidence for moderate-to-severe diarrhea during 2007-2011 delivered from the surveillance and the case-control study were 9.85 episodes in infants (0-11 months), 7.73 in children aged 12-23 months and 2.10 per 100 CYAR in children aged 24-59 months.
- The risk of acute diarrhea decreased with increasing age (12-23 vs. 0-11 months, IRR= 0.72, 95%CI: 0.67-0.77; 24-59 vs. 0-11 months, IRR= 0.10, 95%CI: 0.10-0.11; $p<0.001$).

Case control study

- During the 4-years study period, fecal samples of 784 children aged <5 years with moderate-to-severe diarrhea and 1,545 matched children with no diarrhea were analyzed.
- At least one enteropathogen in 666 (85%) children with moderate-to-severe diarrhea and in 1214 (76%) of the controls; and two or more agents in 376 (48%) cases and in 596 (37%) controls ($p<0.001$) were indentified.
- The etiologic agents detected more frequently included rotavirus, *G. lamblia*, *Cryptosporidium*, EAEC aatA, and *E. hystolyca*.

- Rotavirus and *Cryptosporidium* were significantly associated with moderate-to-severe diarrhea in infants, while ETEC ST (ST only or ST/LT), *Shigella*, adenovirus 40/41 and rotavirus were associated to moderate-to-severe diarrhea in children aged 12-23 months. No association of any specific pathogen with moderate-to-severe diarrhea in the older age group could be confirmed.
- There was a negative interaction between Rotavirus and ETEC ST (ST only or ST/LT): OR= 0.01 (95% CI: 0.00-0.11) in children aged 12-23 months and paradoxically, *G. lamblia* was consistently associated with a lower risk of moderate-to-severe diarrhea in all age groups.
- With the identification of the pathogens independently associated with moderate-to-severe diarrhea, we estimated that 54.0-64.4% of all moderate-to-severe diarrhea episodes could not be attributable to any of the pathogens isolated.
- Rotavirus accounted for more than a third of MSD cases in infants, and its incidence rates markedly exceeded those of others pathogens.

Conclusions

- Over the last decade the incidence of acute diarrhea has dropped by about 80% in Manhiça, Southern Mozambique. Nevertheless, diarrheal disease remains a major cause of morbidity in children aged less than five in Manhiça district.
- Rotavirus, *cryptosporidium*, *Shigella*, ETEC ST and Adenovirus 40/41 were the most important causes of moderate-to-severe diarrhea. Thus, well-known preventive strategies including accelerating the introduction of the effective rotavirus vaccine should be promoted on a wider scale to reduce the current diarrheal diseases burden.
- Our data evidence that HIV infection may be implicated as a huge contributor to *cryptosporidium* prevalence and diarrheal severity in the area.

- The characteristics of the pathogens (pathogenicity, duration of excretion and interaction with other pathogens), host and environmental factors must be explored in greater depth when studying diarrheal etiology, to better understand pathogen causality.

9.3 Article 3

Diarrheal disease in rural Mozambique. Part II: Risk factors of moderate-to-severe diarrhea among children aged 0-59 months

Results

- We investigated the risk factors associated with moderate-to-severe diarrhea in 784 children with diarrhea and 1,545 controls aged 0-59 months.
- The vast majority (94% for cases vs. 97% for controls) of the children had the mother as the primary caretakers, and those children whose primary caretaker was not the mother had a significantly higher risk of developing moderate-to-severe diarrhea compared to those whose primary caretaker was the mother.
- More than two thirds (76% vs. 73%) of the primary caretakers were on the group of lower educational level but this seemed not to be associated with an increased risk of MSD.
- The relation between moderate-to-severe diarrhea and socio-economic status was examined through the ownership of different variables including (cases vs. controls): agricultural land (83% vs. 90%), telephone/mobile (74% vs. 78%), cement floors in the house (67% vs. 71%), radio (44% vs. 46%) and electricity (22% vs. 27%). However, with the exception of ownership of agricultural land, economic indicators used at the household level have not been associated with moderate-to-severe diarrhea risk.
- The main sources of water to the study population were public tap (36% for cases vs. 31% for controls) and borehole (25% for cases vs. 24% for controls). But more than the lack of access *per se*, it was the lack of consistency or regular use of the supplies that was associated with increased risk of moderate-to-severe diarrhea.

- Only a small proportion (6-9%) of the caretakers reported that they used to treat drinking water and among these, the use of chlorine (55% for cases vs. 75% for controls) and boiling water (45% for cases vs. 25% for controls) were the most used methods. Nevertheless, regular water treatment was associated with increased risk of moderate-to-severe diarrhea.
- Most households used traditional pit toilet, (about 90% of cases and 92% of controls) rather than improved facilities such as improved latrines. However, children living in households using an improved facility were at increased risk, and this may reflect incorrect use or insufficient hygiene measures.
- Hand washing appears as a strong protective factor against MSD.
- There were no differences between the cases and the controls with respect to breastfeeding practices in the youngest group (0-11 months). Children aged 12-23 months, who were partially breastfed (OR=0.46, CI; 0.30-0.70) or exclusively breastfed (OR=0.82, CI; 0.51-1.33, $p<0.001$) had a lower risk of moderate-to-severe diarrhea compared to those not breastfed.
- The mean height-for-age z-score among both cases and controls was considerably below the WHO reference for all age groups and, with one exception, deviated further from the reference at older ages (12-59 months), however, no effect on the risk of moderate-to-severe diarrhea was found.

Conclusion

- The results of this study demonstrate that while communities undergo economic development, it may be possible to markedly accelerate the reduction of the burden of diarrheal diseases through aggressive implementation of simple, inexpensive and cross-cutting interventions which promote healthy behavior such as hands washing, safe human waste disposal and breastfeeding practice.

9.4 Article 4

Healthcare utilization and attitudes survey in cases of moderate to severe diarrhea among children aged 0-59 months in the District of Manhica, Southern Mozambique

Results

1st survey

- A total of 1,062 primary caretakers were interviewed and among these 64% of the primary caretakers belonged to the group of lower educational level.
- Ownership within aggregates of different variables defining the level of socioeconomic status included: cement floors (84%), telephone/ mobile (60%), radio (50%), electricity (20%), bicycle (19%), television (18%), refrigerator/freezer (10%) and car/ truck (4%). A fourth of the interviewed caregivers (25%) reported living in precarious conditions of extreme poverty.

Attitudes and perceptions of diarrheal illness

- The vast majority of the caretakers identified findings such as blood in stool (97%), stool increased frequency or decreased consistency (96%), vomiting (95%), rice watery stools (94%) or the presence of dehydration (93%) as markers of severity accompanying a diarrheal episode.
- The clearly identified manifestations of dehydration were the presence of sunken eyes (43%), thirst (43%), wrinkled skin (34%), decreased urinary frequency (33%), and lethargy (29%).
- Washing hands (45%), clean food or water (39%) and proper disposal of human waste (26%) were well known measures to prevent diarrhea.

Diarrheal episode and health care seeking behavior

- 67 caregivers (representing 4% of the DSS population) reported at least one episode of diarrhea during the recall period of two weeks and of these 21 (25%) were considered moderate-to-severe diarrhea.
- Of those reporting diarrhea during the recall period, 41 (65.2%, 95%CI: 51.9-78.4) of the primary caretakers reported seeking care at sentinel health facilities and this proportion increased to 85.9 % (95%CI: 69.6-102.1) among those with moderate-to-severe diarrhea.
- Health facilities were the main sources of care-seeking outside home (94%).
- Other sources of care-seeking included: pharmacy (10%), directly buying medicines at shop/market (2%) or using a traditional healer (1%).
- Health care utilization rose with increasing age: 58.8% (95%CI: 41.8-75.9) for children aged 0-11 months, 60.0% (95%CI: 39.8-80.2) children aged 12-23 months and 79.2% (95%CI: 46.1-112.2) for children aged 24-59 months.
- Independent risk factors for seeking care in health facilities in the first survey included fever (OR=4.69, IC95%; 1.25-17.52, p=0.022) and “not knowing any sign of dehydration” (OR=15.08, IC95%; 1.56-145.43, p=0.020), while having television at home (OR=0.21, IC95%; 0.05-0.84, p=0.029) was related with an independent decreased use of the health facilities.
- There was no association between consultations at a health care structure and the level of education of the caretaker, distance to health structure, adequate knowledge by the caretakers of the manifestations that define severe diarrheal disease, or adequate understanding of the necessary preventive measures against diarrhea.

Diarrheal treatment

- Before going to hospital 43% of the children did not receive any treatment while 30% received herbal medication and 27% received oral rehydration salt.

- When enquired about “how much was offered to the child to drink during the diarrhea illness,” it was found that 11 (12%) of the mothers reduced or stopped their child’s usual liquid/breast milk intake, 47 (73%) maintained the usual amount of liquid or breastmilk, and only 9 (16%) of the mothers gave an increased amount of liquid or breastmilk to their children with diarrhea. Less increasing liquid intake during a diarrheal episode was more markedly noticeable in the youngest age group (infants).

2nd survey

- A total of 2,854 households were included in the analyses and of these 246 caregivers (representing 7% of the DSS population) reported at least one episode of diarrhea during the recall period of two weeks.
- Of those reporting diarrhea during the recall period, 21 (41.5%, 95%CI: 25.9-57.1) in the first round, 36 (44.7%, 95%CI: 32.1-57.2) in the second round, 11 (43.6%, 95%CI: 22.1-65.1) in the third round and 41 (44.0%, 95%CI: 32.9-55.1) in the fourth round used the health structures.
- Health utilization increased in case of moderate-to-severe diarrhea to 51.8% (95%CI: 25.9-77.7) in the first round, 59.0% (95%CI: 41.6-76.5) in the second round, 70.6% (95%CI: 25.2-116.0) in the third round and 54.2% (95%CI: 35.5-73.0) in the fourth round.
- Overall health care utilization was 43.8% (95%CI: 36.9-50.6) and 56.9% (95%CI: 46.2-67.7) for total diarrhea and moderate-to-severe diarrhea, respectively.
- The use of health services was significantly associated to diarrhea with fever (OR=1.88, IC95%; 1.01-3.51, p=0.046) and vomiting (OR=2.78, IC95%; 1.53-5.08, p<0.001).
- Health facilities were the mains sources of care-seeking outside home (86%).

- Other sources of care-seeking outside home included traditional healer (4%), directly buying medicines at shop/market (3%), pharmacy (2%) and unlicensed practitioners (2%) and they were not significantly associated to any diarrheal disease
- When enquired about “how much was offered to the child to drink during the diarrhea illness,” it was found that the majority (79%), of the mothers reduced or stopped their child’s usual liquid/breast milk intake, while 1% maintained the usual amount of liquid or breastmilk, and only 20% gave an increased amount of liquid or breastmilk to their children with diarrhea.

Conclusions

- Community knowledge of the disease, its manifestations and the risk factors associated to severity seems adequate, contrarily to those regarding best practices to treat such episodes, such as for instance the recommendation of increasing liquid intake.
- Despite health access challenges in a rural area such as Manhiça, health services are used regularly from an early age by almost half of the children in their first year of life.
- The use of national health services in case of diarrhea in children under 5 years is fundamentally associated with the perceived need, lower knowledge of dehydration signs and may be hampered by the economic status.
- The other sources of care seeking were not significantly associated with any diarrhea, suggesting that the population considers DSS healthcare as the primary source of treatment in case of diarrheal illness.

- The continuous prospective monitoring realized in the second survey revealed lower prevalence of healthcare use compared to that of the cross-sectional study in the first survey.
- The establishment of continuous prospective monitoring is useful in allowing accounting for changes in health care utilization that may occur due to seasonality or “secular events” and may help to improve health planning for health services utilization.

9.5 Article 5

Severe Malnutrition among Children under the age of 5 admitted to a rural District Hospital in Southern Mozambique

Results

- During the 10 year-long study surveillance, 274,813 children belonging to Manhiça's DSS were seen at the outpatient clinic of Manhiça's District Hospital.
- Almost half of all visiting children (47 %; 128,652/274,813) suffered from some degree of malnutrition (mild, moderate or severe).
- Six per cent (17,188/274,813) of all out-patients could be classified according to their weight-for-age as severely malnourished, but only a small proportion (14.6%; 2,522/17, 188) ended up being admitted.
- Recurring admissions in malnourished children were frequent. Indeed, from the 2,522 severe malnutrition episodes, 1,576 were of children admitted once, 281 were of children admitted twice, 62 of children admitted three times, and further 38 of children admitted more than three times.
- The prevalence of severe malnutrition was 11% (626/5,672) among hospitalized infants (less than 12 months), 23% (1169/5,158) for children aged 12-23 months, 14% (449/3,169) for children aged 24-35 months and 10% (278/2,844) for children 36 to 59 months of age ($p < 0.001$).
- The case fatality rate for patients with severe malnutrition was 7.1% (162/2,274). It was significantly higher in the younger age groups: 12% (65/545) for infants, 6% (61/1068) for children aged 12–23 months and 5% (36/661; $p < 0.001$) for those aged 24 months.
- In the adjusted analysis, invasive bacterial disease (OR=2.37; 95% CI, 1.46-3.84), hypoglycaemia (OR=4.67; 95% CI, 2.53-8.60), oral candidiasis (OR=3.72; 95% CI,

2.19-6.34), edema (OR=2.80 95% CI 1.64-4.77), pallor (OR=1.81; 95% CI, 1.10-2.96), deep breathing (OR=2.71; 95% CI, 1.43-5.14) and acute diarrhea (OR=1.84; 95% CI, 1.22-2.78) were independently associated with an increased risk of in-hospital mortality among severely malnourished children, while malaria parasitaemia (OR=0.58; 95% CI, 0.37-0.90) and increasing age (OR=0.58; 95% CI, 0.36-0.82 and OR=0.45; 95% CI, 0.25-0.82 for 12-23 and 24-59 months age group respectively) were independently associated with a lower risk of a poor outcome.

- During the study period, minimum community-based incidence rates (MCBIRs ; calculated only for children living in DSS area) of severe malnutrition in the study area declined significantly from 33 in 2001 to 7 cases/1000 child-years in 2010 ($p < 0.001$).
- MCBIRs were estimated to be 20/1,000 CYAR in children aged 0–11 months, 35/1,000 CYAR in children aged 12–23 months and 7/1,000 CYAR in children aged 24- 59 months ($p < 0.001$).

Conclusions

- Despite the important decline tendency, severe malnutrition among admitted children in this area of Mozambique is common, highly associated to the risk of death but frequently undetected.
- Measures to improve its recognition by clinicians responsible of the first evaluation of patients at the outpatient level are urgently needed so as to improve their likelihood of survival.
- The rapid management of complications such as hypoglycaemia and concomitant co-infections such as bacteremia, acute diarrhea, oral candidiasis and HIV/AIDS may

contribute to reverse the intolerable toll that malnutrition poses in the health of children in rural African settings.

- The fact that recurring admissions in malnourished children were frequent, demonstrate that improvements in severe malnutrition incidence rely not only on the availability of a well-functioning and accessible public health system, but also on parallel enhancements in the community's socio-economic status.

10. GENERAL CONCLUSIONS

1. Despite the predominantly decreasing trend of the incidence of diarrheal disease in rural Mozambique, diarrheal diseases remain a major cause of morbidity and mortality among children aged less than five years age, similarly to what can be seen in other developing countries,.
2. Rotavirus, *cryptosporidium*, *Shigella*, ETEC ST and Adenovirus 40/41 are the most important causes of diarrheal disease among children less than five years of age.
3. An urgent need exists to accelerate the introduction of rotavirus vaccination and to simultaneously improve the implementation of existing interventions with proven effectiveness, such as adjunct treatment of acute diarrhea with zinc and to revitalize diarrheal disease case management algorithms shown to reduce malnutrition.
4. The substantial burden of moderate-to-severe diarrhea and its close association to malnutrition show that preventive strategies targeting as few as five pathogens could potentially reduce this disease and its associated *sequelae* by about 40% during the first 2 years of life
5. Simple, inexpensive and cross-cutting interventions which promote healthy behavior such as hand washing, safe human waste disposal and breastfeeding practice may also accelerate reduction of the burden of diarrheal disease in Manhiça district.
6. The knowledge of diarrheal disease, its manifestations and the risk factors associated to severity seems adequate among the community of Manhiça district, contrarily to those regarding best practices to treat such episodes, such as for instance the recommendation of increasing liquid intake.

7. Health access remains challenging for a large proportion of the inhabitants of Manhiça's district, nevertheless national healthcare is still considered the primary source of care/treatment for diarrheal disease affecting children under five years age.
8. The use of national health services in case of diarrhea in children under 5 years is fundamentally associated with the perceived need, lower knowledge of disease and may be hampered by the economic status.
9. Severe malnutrition among admitted children in Manhiça District is common but frequently undetected, despite associating a high risk of death. Thus efforts for malnutrition early recognition may improve the survival likelihood of those children.
10. The rapid management of complications such as hypoglycaemia and concomitant co-infections such as bacteremia, acute diarrhea, and HIV/AIDS may contribute to reversing the intolerable toll that malnutrition poses in the health of children in rural African settings.

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