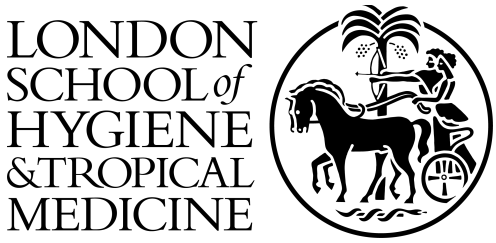


# The effect of water, sanitation and hygiene conditions on enteric pathogen exposure and related health outcomes in vulnerable children – evidence from two trials

**OLIVER CUMMING**

**Thesis submitted in accordance with the requirements**
**for the degree of Doctor of Philosophy of the**
**University of London**

**APRIL 2025**

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Funded in part by the UK Department of International Development
and United States Agency for International Development

## Statement of own work

I, Oliver Cumming, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that it has been indicated in the thesis.

Oliver Cumming

04.04.2025

## Acknowledgements

First and foremost, I thank the participants and their families who supported this research with their time and trust. I thank all the different health workers who made it possible to design and deliver these interventions through existing health system structures. This includes the nurses and health extension workers in Senegal, and the Community Health Workers and Community Health Volunteers in Kenya. The highlight of this work for me was sharing the findings with the families whose children participated in this research as well as the health workers who delivered the interventions. I would also like to acknowledge and thank the Department of Health of the Country Government of Kisumu and the Ministry of Health for Senegal for supporting this work.

Researchers enjoy research for very different reasons. For me, the greatest pleasure lies in the opportunity for real and meaningful exchange of ideas with colleagues. I can't list everyone to who I owe thanks, but I must name a few. In Kisumu, first and foremost my collaborator and friend, Dr Jane Mumma – thank you, Jane! Also, Dr Sheillah Simiyu and Eva Aseyo, in particular, but also the whole Safe Start team at GLUK. And, Dr Kelly Baker for her good humour and patience as we found our way to finish line. For the work in Senegal, I thank the whole TISA team but especially Matar Ba, Emile Cabo, Dr Françoise Siroma, Dr Alou Traoré, and Alex Devort. Only two people stayed the course, from the very beginning to the very end – and for every bump in the road between – and they were Dr Dieynaba N'Diaye and Dr Antonio Vargas Brizuela. Dieynaba and Antonio, thank you for the camaraderie and solidarity these past years.

My home at LSHTM is the Environmental Health Group (EHG) and I cannot imagine a better place to do research nor a better group of people to do research with. Work feels less like work when you are surrounded by smart fun people with a shared passion for research and a commitment to improving public health. There are three people in EHG in particular who I thank: Prof. Robert Dreibelbis, Dr Jackie Knee and Dr Laura Braun.

Without their support across the many projects we share, I don't know how I would have found the time to get this done.

I have had two supervisors in this long journey who ran this (long) race as a relay event. Prof. Sandy Cairncross persuaded me to come to LSHTM and then persuaded me to start a PhD. From meetings in his office to reading his many elegant research papers, Sandy taught me that good research requires two things rare commodities: humility and curiosity. It was Prof. Tanya Marchant who – after a long hiatus - persuaded me to complete my PhD. She seemed so convinced I could get it done that magically I got it done. I really can't thank you enough, Tanya.

And finally, the most important people in my life. For William, Lucy and Samuel, I am sorry for all the missed bike rides, climbing trips, and outings with Undur but it's done now so we can get back to the important stuff.  And, Elizabeth, without you, this and everything else would not be possible. Thank you.

## Abstract

Gastro-intestinal infection by bacteria, viruses, protozoa and soil-transmitted helminths inflict a heavy burden of disease, especially among young children living in settings without safe water, sanitation and hygiene (WASH) services. Historically, it has been the associated diarrhoeal disease burden that has attracted most attention in global health. There is growing evidence of the harmful effects of asymptomatic enteric pathogen carriage in early life on growth and development. It is unclear whether basic water, sanitation and hygiene interventions are sufficient to prevent exposure to these pathogens and mitigate the adverse health impacts. The aim of this thesis was to assess the effectiveness of targeted WASH interventions in reducing enteric pathogen exposure and related health consequences in two particularly vulnerable populations.

Evidence is drawn from two randomised controlled trials of WASH interventions implemented in two different settings, urban Kenya and rural Senegal. Both interventions were designed with, and delivered by, the health system with the aim of reducing enteric pathogen exposure among vulnerable children by mitigating the risk posed by environmental hazards, such as contaminated drinking water and food. In Kenya, a food hygiene intervention targeted infants in high-density informal urban neighbourhoods of the city of Kisumu. In northern Senegal, a water treatment and hygiene intervention targeted children undergoing outpatient treatment for severe acute malnutrition (SAM) in predominantly low-density rural areas of the departments of Podor and Linguère.

There was evidence for both interventions reducing diarrhoea but there was no effect on enteric pathogen detection in children nor on the other associated outcomes measured, most notably recovery from SAM. The burden of asymptomatic enteric infection in both populations was high and these targeted WASH interventions failed to reduce this. These two trials confirm the importance of reducing enteric pathogen exposure in vulnerable populations but suggest that such limited short-term interventions delivered by the health system are insufficient to address this. The

findings add new evidence to support the ambitious Sustainable Development Goal targets for universal access to safely managed water and sanitation services.

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## List of abbreviations

| | |
|---|---|
| ACF | ACF Action Contre la Faim |
| BHA | BHA Bureau of Humanitarian Assistance |
| CFU | Colony Forming Unit |
| CHC | Community Health Committee |
| CHEW | Community Health Extension Worker |
| CHV | Community Health Volunteer |
| CNERS | CNERS Comité National d'Ethique pour la Recherche de Senegal |
| cRCT | Cluster Randomized Control Trial |
| DHS | Demographic Health Surveys |
| FCDO | Foreign Commionwealth and Development Office |
| GEMS | Global Enteric Multicenter Study |
| GLUK | Great Lakes University of Kisumu |
| GPS | Global Positioning System |
| ICC | Intraclass Correlation Coefficient |
| ICP | Infirmier Chef de Poste, ie head nurse |
| LMIC | Low- and Middle-Income Countries |
| IRB | Institutional Review Board |
| LSHTM | London School of Hygiene and Tropical Medicine |
| MCD | Médecin Chef de District |
| MICS | Multiple Indicator Cluster Survey |
| MSD | Moderate and Severe Diarrhoea |
| PCMA | Prise En Charge Communautaire De La Malnutrition Aiguë |
| PCMAS | Prise en Charge de la Malnutrition Aiguë et Sévère |
| cRCT | Cluster randomised controlled trial |

| | |
|---|---|
| RUTF | Ready-to-Use Therapeutic Food |
| SAM | Severe Acute Malnutrition |
| TISA | Traitement Intégré de la Sous-nutrition Aiguë |
| UCAD | Université Cheikh Anta Diop De Dakar |
| UREN | Unité de Réhabilitation et d'Education Nutritionnelle i.e. health posts |
| UNICEF | Fonds des Nations Unis pour l'Enfance |
| WASH | Water, Sanitation and Hygiene |
| WHO | World Health Organisation |
| WHZ | Weight for Height Z-score |

# BACKGROUND

# 1

# Chapter 1: Thesis Overview

## Thesis conception

The original motivation for this PhD was to understand how exposure to pathogens contributed to chronic childhood undernutrition, how water, sanitation and hygiene (WASH) interventions have often failed to prevent these exposures, and how interventions might be more effectively designed and delivered to mitigate this. I originally focused my doctoral research on chronic undernutrition, with childhood growth faltering as the primary outcome of interest. Early in my PhD I undertook a literature review to assess the evidence for the effect of WASH on chronic undernutrition and how WASH interventions might be designed to optimise their effectiveness to prevent stunting. This work highlighted the growing evidence for the importance of stool-based enteric pathogen detection, both as an indicator of the effectiveness of WASH interventions in preventing exposure and as determinant of subsequent disease and undernutrition. These insights informed my decision to adopt a more specific focus on enteric pathogen detection in children versus the more distal and multi-factorial outcome of chronic undernutrition. Stool-based enteric pathogen detection using multiplex molecular methods offers many advantages over reported diarrhoea that has dominated the WASH epidemiological literature. Notably these outcomes provide an objective measure of past exposure, allow the simultaneous detection of multiple pathogens, lie unambiguously on the causal path to disease, and their detection – whilst predominantly asymptomatic – may be a stronger predictor of growth faltering than diarrhoea.

In line with the narrower focus on enteric pathogen exposure I considered two different WASH interventions. Both interventions were co-designed with local health actors and delivered through the existing policy and structures of the local health systems. The interventions were both designed to reduce enteric pathogen exposure at a critical moment within a vulnerable population within each context. In Kenya, the intervention was an infant food hygiene intervention, delivered in a complex peri-urban/urban setting combining high population density with poor environmental conditions. The intervention was designed to reduce enteric pathogen exposure among infants during the weaning period. In Senegal, the intervention was water treatment and hygiene

intervention delivered in low density rural settings with limited healthcare access and designed to limit enteric pathogen exposure among children being treated on an outpatient basis for severe acute malnutrition. The thesis draws on the results of two trials to assess the effectiveness of these WASH interventions in reducing enteric pathogen exposure and other related outcomes.

## Research Context

The research presented in this thesis took place within two trials, the Safe Start trial in Kenya and the TISA (Traitement Integré pour la Malnutrition Aiguë Sévère) trial in Senegal. The methodology for the two trials are described in detail in Chapters 4 and 5, and the main study characteristics presented in Table 1 using the Population, Intervention, Comparison, Outcome and Design (PICOD) criteria developed by the Cochrane Collaboration (1) .

From a study design perspective, the two trials have a number of common features which facilitate a synthetic discussion of their results. They are both cluster-randomised controlled trials where the cluster is formed by a unit of the health system that is meaningful for the intervention. In the Safe Start trial the clusters represent the catchment areas for the Community Health Volunteers (CHVs) who delivered the intervention and operate within discrete geographic areas thereby limiting the risk of contamination between arms. In the TISA trial, the clusters represent the catchment areas for primary health care centres (Unités de Récupération d'Education Nutritionnelle [UREN]) providing outpatient care for uncomplicated cases of severe acute malnutrition (SAM). Both trials also included an active control arm to limit the risk of confounding that might arise due to the differing levels of interaction with health system agents between arms versus any effect that might result from the intervention itself. In the Safe Start trial, participants in the control group received an equal number of household visits by CHVs as those in the intervention group and in the TISA trial participants in the control group had the same number of health centre visits as those in the intervention group.  Lastly, whilst other health outcomes were included in the

trials, both studies included the same three related outcomes: stool-based enteric pathogen detection, diarrhoea, and all-cause mortality. Although the collection methods differed for the stool sample collection (described in full in chapters 4 and 5) both used the same multiplex molecular methods for detection of enteric pathogens.

My role in these studies was Principal Investigator (PI). In my professional capacity as a researcher at the London School of Hygiene and Tropical Medicine, I received research grants from the United Kingdom's Foreign Commonwealth and Development Office (FCDO) and the Bureau for Humanitarian Assistance (BHA) of the United States Agency for International Development (USAID) for these studies. As the PI, I led the conception, design and implementation of both studies, working in collaboration with investigators in the partner organisations. Both trials were registered at clinicaltrials.gov registry in advance of the enrolment of any participants.

Table 1.1 - Comparison of PICOD criteria for the two trials

| | **Safe Start** | **TISA** |
|---|---|---|
| **Population** | Children aged 6-9 months of age<br><br>Resident in informal peri-urban neighbourhoods of Kisumu, Kenya<br><br>Children registered with Community Health Volunteers | Children aged 6 – 59 months of age<br><br>Resident in predominantly rural districts of northern Senegal<br><br>Children admitted as non-complicated SAM cases to outpatient treatment |
| **Intervention** | Infant food hygiene:<br><br>• 20 L water container with tap | Water treatment and hygiene:<br><br>• 20 L water container with tap |

| | | |
|---|---|---|
| | • 500 mL liquid soap<br>• Interactive sessions with CHV<br>• Calendar with messaging<br>• Infant feeding utensils<br>• Sealable food containers<br>• Calendars and place mat | • 3 bars of soap<br>• Aquatabs for 20 L per day<br>• Instruction sessions with nurse<br>• Instruction leaflet |
| **Comparison** | Active control group<br><br>Equal number of visits from Community Health Volunteers delivering standard public health information | Active control group<br><br>Equal level of contact with nurses delivering the standard of care for outpatient treatment of severe acute malnutrition |
| **Outcomes** | 1. Enteric pathogen detection<br><br>2. Diarrhoeal disease<br><br>3. All-cause mortality | 1. Enteric pathogen detection<br><br>2. Diarrhoeal disease<br><br>3. All-cause mortality<br><br>4. Recovery from SAM<br><br>5. Weight gain<br><br>6. Referral to tertiary care |
| **Study design** | Cluster-randomised controlled trial | Cluster-randomised controlled trial |

| | | |
|---|---|---|
| | Pragmatic design with intervention delivered through existing health system structures | Pragmatic design with intervention delivered through existing health system structures |

## Thesis Aim and Objectives

The aim of this thesis was to assess the effectiveness of WASH interventions in reducing enteric pathogen exposure and improving related health consequences in two vulnerable populations.

The specific objectives were:

1. Synthesise current evidence linking water, sanitation and hygiene to undernutrition and identify entry points for nutrition sensitive WASH interventions

2. Assess the burden of enteric pathogen detection in two different vulnerable populations

3. Evaluate the effect of an infant food hygiene intervention on enteric pathogen detection and related health outcomes in a complex urban environment

4. Evaluate the effect of a water treatment and hand hygiene intervention on enteric pathogen detection and related health outcomes among children with severe acute malnutrition

## Thesis components

This thesis comprises nine chapters, covering the following: (i) an introduction, including an overview of the thesis (Chapter 1) and a review of relevant literature that has motivated and informed the research (Chapters 2 and 3); (ii) the methods for research conducted (Chapters 4 and 5); the research results (Chapters 6, 7 and 8); and the general discussion and conclusions of the thesis (Chapter 9). Two chapters (2 and 4) have already been published as peer-reviewed articles so are included in research

paper style format with a cover sheet. The results chapters (6-8) have been prepared for submission to peer-reviewed journals and are therefore all also presented in a research paper style format with cover sheet. The table below summarises the content of each of the chapters.

Table 1.2 - Summary of thesis chapters

| **Chapter** | **Summary of content** |
|---|---|
| Chapter 1 | This chapter provides an overview of the thesis. Firstly, I describe the conception of the research and the decision to focus on enteric pathogen exposure. The section on research context describes the two trials on which the thesis is built, explaining what is common between them and what differs. I then set the aim and specific objectives of the thesis before summarising each chapter. |
| Chapter 2 | This chapter is the first of two chapters which serve as the background for the thesis. At the inception of my PhD, I set out to investigate the relationship between WASH and chronic undernutrition or stunting. In this chapter which has been published in a peer-reviewed journal (2), I address two broad questions relating to that original area of focus: (1) can WASH interventions make a significant contribution to reducing the global prevalence of childhood stunting, and (2) how can WASH interventions be delivered to optimize their effect on stunting and accelerate progress? Based on the conclusions of this review I decided to narrow my focus to the issue of enteric pathogen exposure. |
| Chapter 3 | This chapter serves as the second part of the background of the thesis and builds on the previous chapter. I supplement the earlier literature review that focused primarily on stunting to go further in reviewing the evidence concerning the burden, consequences and relationship to WASH services of enteric pathogen detection in children. As the earlier |

| | |
|---|---|
| | review was completed at the point of transition between the Millennium Development Goals (MDGs) and the Sustainable Development Goals (SDGs) I discuss this transition from the MDGs to the SDGs and the implications for the study of WASH-related enteric pathogen exposure. |
| Chapter 4 | This chapter is the first of two chapters which serve as the methods section of the thesis. In the first of these, I present the rationale and methods for the Safe Start trial which was a cluster-randomised controlled trial in Kisumu, Kenya to evaluate the effect of a food hygiene intervention on enteric pathogen detection and diarrhoea among infants. This chapter describes the setting and the process by which the intervention was co-designed with communities and health system actors through a process of formative research and dialogue. This chapter has been published in a peer-reviewed journal (3). |
| Chapter 5 | In this chapter, I present the rationale and methods for the TISA trial which was a cluster-randomised controlled trial in Senegal to evaluate the effect of integrating a water treatment and hygiene promotion intervention in the standard of care for outpatient treatment of children with uncomplicated severe acute malnutrition (SAM). The outcomes for this trial included enteric pathogen detection and diarrhoea as well as outcomes relating to SAM recovery. |
| Chapter 6 | This chapter if the first of three chapters (6-8) which serve as the results section of the thesis. In this chapter I describe the results of a cross-sectional study conducted at the baseline of the Safe Start trial to assess the prevalence of enteric pathogen detection and the diversity of pathogens detected among six-month year-old infants. I also assess the association between pathogen detection and symptomology (diarrhoea), and the clustering of pathogens within infants. This chapter |

| | |
|---|---|
| | is presented as a research paper with cover sheet as it will be submitted for publication in a peer-reviewed journal. |
| Chapter 7 | In this chapter I present the results of the Safe Start trial. The results include the effects on enteric pathogen detection and diarrhoea but also report on the delivery of the intervention and response of caregivers to the intervention. This chapter is presented as a research paper with cover sheet as it will be submitted for publication in a peer-reviewed journal. |
| Chapter 8 | In this chapter I present the results of the TISA trial. The results include the effects of the intervention on enteric pathogen detection and diarrhoea as well as outcomes related to the outpatient treatment of SAM, including recovery, referral to tertiary healthcare and weight gain. This chapter is presented as a research paper with cover sheet as it will be submitted for publication in a peer-reviewed journal. |
| Chapter 9 | This is the concluding chapter of the thesis. I discuss the main findings as they relate to the aim and objectives for this thesis, as well as the limitations of the research. Finally, I consider the implications for policy and future research. |

# 2

## Chapter 2: Water, Sanitation and Hygiene, and Stunting

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# RESEARCH PAPER COVER SHEET

**Please note that a cover sheet must be completed for each research paper included within a thesis.**

## SECTION A – Student Details

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| **First Name(s)** | Oliver | | |
| **Surname/Family Name** | Cumming | | |
| **Thesis Title** | The influence of water, sanitation and hygiene conditions on enteric pathogen exposure and related health outcomes among vulnerable children – evidence from two trials | | |
| **Primary Supervisor** | Prof. Tanya Marchant | | |

**If the Research Paper has previously been published please complete Section B, if not please move to Section C.**

## SECTION B – Paper already published

| | | | |
|---|---|---|---|
| Where was the work published? | Journal of Maternal and Child Nutrition | | |
| When was the work published? | 17.05.2016 | | |
| If the work was published prior to registration for your research degree, give a brief rationale for its inclusion | n/a | | |
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|---|---|
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| | |
|---|---|
| Stage of publication | Choose an item. |

**SECTION D – Multi-authored work**

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|---|---|
| For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary) | I conceived the review, conducted the literature review and prepared the first draft. My supervisor (Prof Sandy Cairncross) reviewed and provided input on the initial draft before finalisation and submission to the journal. Following peer review, I responded to reviewers and revised the manuscript accordingly. |

**SECTION E**

| | |
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| **Student Signature** | [redacted] |
| **Date** | 3[redacted] |

| | |
|---|---|
| **Supervisor Signature** | [redacted] |
| **Date** | 31.01.2025 |

Maternal & Child Nutrition

DOI: 10.1111/mcn.12258

Review Article

# Can water, sanitation and hygiene help eliminate stunting? Current evidence and policy implications

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**Abstract**

Stunting is a complex and enduring challenge with far-reaching consequences for those affected and society as a whole. To accelerate progress in eliminating stunting, broader efforts are needed that reach beyond the nutrition sector to tackle the underlying determinants of undernutrition. There is growing interest in how water, sanitation and hygiene (WASH) interventions might support strategies to reduce stunting in high-burden settings, such as South Asia and sub-Saharan Africa. This review article considers two broad questions: (1) can WASH interventions make a significant contribution to reducing the global prevalence of childhood stunting, and (2) how can WASH interventions be delivered to optimize their effect on stunting and accelerate progress? The evidence reviewed suggests that poor WASH conditions have a significant detrimental effect on child growth and development resulting from sustained exposure to enteric pathogens but also due to wider social and economic mechanisms. Realizing the potential of WASH to reduce stunting requires a redoubling of efforts to achieve universal access to these services as envisaged under the Sustainable Development Goals. It may also require new or modified WASH strategies that go beyond the scope of traditional interventions to specifically address exposure pathways in the first 2 years of life when the process of stunting is concentrated.

*Keywords*: sanitation, water, stunting, child nutrition, child public health, early growth.

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## Introduction

This article was inspired by the 'Stop Stunting' Conference held in Delhi last year to convene actors from multiple countries and sectors to address a shared concern: the enduring and seemingly intractable challenge of childhood stunting in South Asia. Huge progress has been made in much of the South Asia region in extending healthcare, education and economic opportunity, and these investments have brought dramatic improvements in maternal and child mortality, in school retention rates and in overall economic output. Despite this laudable progress, the prevalence of childhood stunting in South Asia remains high with profound consequences for those children affected: increasing their susceptibility to infectious disease morbidity and mortality, diminishing their future educational achievements and reducing their economic productivity in later life. The failure to address stunting in South Asia, and other high-burden regions, stands to undermine progress in other sectors and trapping future generations in poverty and ill health.

Stunting is a complex problem as depicted by various conceptual frameworks, focused on 'child malnutrition' (UNICEF 1990), 'maternal and child undernutrition' (Black *et al.* 2013) and 'food and nutrition security' (Gross *et al.* 2000). The causes of stunting are multifactorial and inter-linked, spanning biological, social and environmental spheres. Water, sanitation and hygiene (WASH), the focus of this paper, feature at various levels in these frameworks with varying degrees of proximity to the outcome of stunting, as immediate or proximate risk factors but also as more distant causes or determinants of stunting. For example, different aspects of WASH have been plausibly linked to all four 'pillars' of the food and nutrition security framework (Cumming *et al.* in press): food 'availability', through water as a resource

 

for agricultural production; food 'access', through household income diverted from food by the cost of obtaining water and ensuring adequate sanitation; food 'stability', through the economic shock of treating related infectious disease or associated inability to work; and lastly food 'utilization', through the effect of WASH-related enteric infections on the body's ability to utilize the available nutrients.

Two broad questions emerge for those considering WASH as a potential component of more effective comprehensive strategies to address stunting. Firstly, can WASH interventions make a significant contribution to reducing the global prevalence of childhood stunting? Secondly, and if so, how can WASH interventions be delivered to optimize their effect on stunting and accelerate progress? These questions are of importance to both the WASH and nutrition sectors, and for wider debates concerning the allocation of scarce resources available for improving public health and other social outcomes in low and middle-income countries where the burden of stunting is highest.

Here, we review how poor water, sanitation and hygiene can influence the process of stunting through biological and social mechanisms and then consider the strength of evidence available for an effect of these interventions on stunting. Secondly, we identify the underlying parameters that might plausibly govern the degree to which WASH interventions reduce the risk of stunting and then discuss the implications for practitioners and policymakers concerned with mobilizing WASH resources in support of broader efforts to reduce stunting.

## Water, sanitation and hygiene

The importance of safe drinking water, sanitation and hygiene (WASH) has long been recognized with regard to public health in general and the health of infants and young children in particular (Jones 1923). Indeed, the birth of 'public health' as a defined area of public policy and as a professional discipline is now synonymous with these endeavours to improve 'sanitary conditions', following the pioneering work of Chadwick (1842), Farr (1866) and Snow (1855) in the 19th century. WASH is often divided into four rather than three categories, with 'water' interventions divided into two subcategories: 'water quantity' and 'water quality'. The former describes interventions that improve the quantity of drinking water available to the household, and the latter describes interventions that improve the microbial quality of drinking water, whether this is at the water source or at the point of use or consumption. Sanitation concerns technologies and behaviours that serve to safely contain excreta, preventing human contact, and hygiene is commonly used to mean washing with soap at critical times (e.g. after defecation and before eating).

These public health interventions together form an interlocking set of barriers that prevent exposure to disease-causing organisms via five transmission pathways as famously depicted in the 'F-diagram' (Fig. 1) of Wagner & Lanoix (1958). The interdependency of these barriers is well illustrated by the cholera outbreak investigated by John Snow in Soho, London, almost two centuries ago (Snow 1855). The index case was an infant whose infected stools were emptied into a poorly constructed cesspool,

**Key messages**

- Water, sanitation and hygiene (WASH) remain critical interventions for improving maternal and child health.
- A growing body of evidence suggests that WASH are important determinants of childhood stunting.
- WASH interventions influence stunting through multiple direct biological mechanisms and by various social and economic mechanisms.
- There is sufficient evidence to justify the inclusion of WASH within national and international strategies to reduce stunting.
- To address stunting WASH policy and programmes should explicitly address exposures in early childhood.

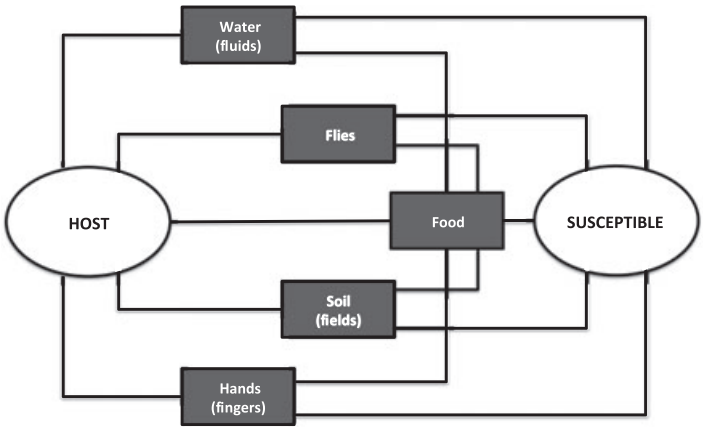


**Fig. 1.** The 'F-diagram'. Source: Adapted from Wagner & Lanoix1958 and Kawata 1978.

which contaminated the water source that served the now infamous Broad Street water pump (Johnson 2008). While Snow's work elegantly demonstrated that cholera was transmitted from host to susceptible individual by the medium of water, the epidemic itself had as much to do with the prevailing sanitation infrastructure and hygiene behaviours as it did with the water supply.

Although WASH interventions are often described in terms of their role in preventing disease transmission, the benefits are not confined to health. Improvements in water supply often serve to reduce the distance travelled to the water source leading to significant time savings for poor households that can transform the lives of the women and children whose responsibility it largely is to collect water [World Health Organization (WHO) & UNICEF 2010]. A senior World Bank economist famously argued that these benefits alone provide sufficient economic justification for the investment costs of water supply without any consideration of the health benefits that may accrue (Churchill *et al.* 1987). The non-health benefits of sanitation include privacy and convenience afforded by improved facilities. There is now a growing literature that documents that this lack of 'privacy and convenience' can lead to an increased risk of violence, whether this is physical, sexual and psychological, that is borne primarily by women. It is perhaps because of these risks that shared and public sanitation facilities have been found to be less preferable to women as compared with men (Biran *et al.* 2011).

## Global coverage for water, sanitation and hygiene

The WHO/UNICEF Joint Monitoring Programme tracks progress against target 7.c of the Millennium Development Goals (MDG): 'to reduce by half the proportion of the population without sustainable access to safe drinking water and improved sanitation by 2015'. At a global level, it has been announced that while the water component of this MDG target was met in 2010, the sanitation target has been missed by a substantial margin.

In most countries defined as low and middle income (LMIC) (Group 2015), most people lack household-level access to a safe and reliable supply of drinking water, and to a safe and acceptable form of sanitation (WHO & UNICEF 2014). Globally, it has been estimated that over one-third of the world's population are without these services at home (Cumming *et al.* 2014). While challenges persist in other regions, sub-Saharan Africa and South Asia account for the greatest deficits in access to safe water and sanitation (WHO & UNICEF 2014). Access to, or more appropriately the practice of, safe hygiene is much harder to estimate and is not currently reported at a global level. The most comprehensive published analysis to date, based on the results of a systematic review of studies reporting observed handwashing practice, estimated that fewer than one in five people globally wash their hands with soap after defecation (Freeman *et al.* 2014b).

Analysis of historical progress and current coverage reveals marked geographic and social disparities in access to these services. Between countries (WHO & UNICEF 2014) but also within many countries (Pullan

*et al.* 2014), access to safe water and sanitation varies significantly. Disparities in access between rural and urban communities are well documented, with access to both water and sanitation services in rural generally much lower than in urban areas, especially in LMIC (Bain *et al.* 2014b). Viewed at the level of mean global averages, the differences between urban and rural areas are striking: in 2012, there were 500 million more people without access to safe water in rural areas vs. urban areas, and 1 billion more without access to sanitation (WHO & UNICEF 2014). However, disparities in access between the poorest quintiles for rural and urban populations are far less marked (Rheingans *et al.* 2013).

More than half of the world's population now reside in urban areas, and over one-third of these urban dwellers live in 'slums or informal settlements' with that proportion being much higher in LMIC Development, W. H. O. C. F. H., & Programme, U. N. H. S. (2010). Although access to safe water and sanitation is generally higher in urban vs. rural areas (Bain *et al.* 2014b), the proportion of the urban population with access to safe services is actually falling as investment fails to keep pace with urban population growth (WHO & UNICEF 2015). It has long been recognized that the risk of enteric infection may be greatest in poor urban areas due to the combination of high population density and limited infrastructure (White *et al.* 1972), which is supported by studies looking at certain soil-transmitted helminth infections (Strunz *et al.* 2014b) and diarrhoea (Mock *et al.* 1993) and childhood undernutrition (Olack *et al.* 2011). A failure to target investments at the growing population living in informal areas may undermine progress on reducing child mortality in some countries (Fotso *et al.* 2007; Rheingans *et al.* 2013).

The global prevalence of childhood stunting has declined considerably during the MDG period: while in 1990 40% of children globally were estimated to be stunted (height for age z-score [HAZ]$< -2$), it is now estimated that this has fallen to below a quarter (Black *et al.* 2013). In absolute terms, the number of children with stunting has fallen by approximately 100 million, although this still leaves 150 million children stunted today (Black *et al.* 2013). As with the shortfall in water and sanitation coverage, the global burden of stunting is heavily concentrated in just two regions of the world: South Asia and sub-Saharan Africa.

## The broader infectious disease burden attributable to WASH

Safe WASH is of paramount public health importance *without* considering the plausible impact on childhood stunting. Improved access to WASH can prevent a large infectious disease burden that includes diarrhoeal diseases but also other important infectious diseases. Diarrhoeal disease, encompassing a broad range of bacterial, viral and protozoal enteric infections, and largely preventable with improved WASH, was ranked as the fourth leading cause of disability globally in 2010, after ischaemic heart disease, lower respiratory heart infections and strokes (Murray *et al.* 2013).

A recent series of papers by a WHO-led group of experts quantified the global diarrhoeal disease burden attributable to poor water, sanitation and hygiene (Bain *et al.* 2014a; Freeman *et al.* 2014a; Prüss-Ustün *et al.* 2014; Wolf *et al.* 2014). The authors estimated that approximately 500 000, 280 000 and 300 000 deaths are attributable to poor water, sanitation and hygiene, respectively (Prüss-Ustün *et al.* 2014). Using a formula for the aggregate burden for a cluster of risk factors (Lim *et al.* 2013), the total diarrhoeal burden of disease for WASH was estimated at over 800 000 deaths, equivalent to 1.5% of the total global burden of disease (Prüss-Ustün *et al.* 2014). Almost half of these deaths were among children, with WASH accounting for 5.5% of the total burden of disease for this age group (Prüss-Ustün *et al.* 2014), and diarrhoea remains a leading cause of child deaths globally and especially in high-burden regions, such as sub-Saharan Africa and South Asia (Liu *et al.* 2012).

Supported by evidence of variable quality, WASH is linked to a wide range of other infectious disease health outcomes, including helminth infections (Ziegelbauer *et al.* 2012; Strunz *et al.* 2014a), schistosomiasis (Grimes *et al.* 2014), trachoma (Stocks *et al.* 2014), respiratory infections (Rabie & Curtis 2006) and maternal and reproductive infections (Benova *et al.* 2014). Aggregating the disease burden for WASH – itself a cluster of overlapping risk factors – to take account of multiple and related outcomes (e.g. diarrhoea and pneumonia) is

methodologically challenging. However, one recent WHO analysis that did this reported that approximately 10% of the total global burden of disease could be prevented with improved WASH (WHO 2008).

## Can safe water, sanitation and hygiene prevent stunting?

The pathways linking poor WASH to childhood stunting are complex, spanning multiple direct biological routes and many broader, less direct routes. To understand these, it is necessary to place the generally better investigated direct biological linkages within a broader socio-economic framework which considers aspects such as accessibility and affordability of water supplies and sanitation facilities. Here, we first consider the biological mechanisms that plausibly link WASH and stunting, and then secondly, we consider the social and economic mechanisms.

### *Biological mechanisms*

Three biological mechanisms, in particular, have been described that link poor WASH to undernutrition directly: (1) via repeated bouts of diarrhoea (Briend 1990; Checkley *et al.* 2008; Petri *et al.* 2008; Richard *et al.* 2013); (2) soil-transmitted helminth infections, *Ascaris lumbricoides*, *Trichuris trichiura*, *Ancylostoma duodenale*, and *Necator americanus* (O'lorcain & Holland 2000; Prüss-Üstün & Corvalán 2006; Hall *et al.* 2008; Ziegelbauer *et al.* 2012); and (3), a subclinical condition of the gut, referred to variously as tropical enteropathy (Baker & Mathan 1972; Humphrey 2009a), environmental enteropathy (Fagundes-Neto *et al.* 1984; Korpe & Petri 2012) or, most recently, and as used here, environmental enteric dysfunction (EED) (Haghighi *et al.* 1997; Humphrey 2009b; Keusch *et al.* 2014; Crane *et al.* 2015). For each of these, the effect of WASH on undernutrition is mediated by exposure to enteric pathogens and symptomatic or asymptomatic infection.

Frequency of diarrhoeal disease, as a syndrome, irrespective of its causes, is strongly correlated with growth faltering (Checkley *et al.* 2003; Checkley *et al.* 2008). Demonstrating a causal relationship between diarrhoea and malnutrition though is challenging, as undernutrition can increase both the likelihood and severity of diarrhoea disease (Brown 2003; Caulfield *et al.* 2004). However, a recent pooled analysis of data from nine countries with longitudinal morbidity and anthropometry provides evidence that repeated bouts of diarrhoea cumulatively increase the risk of stunting in children (Checkley *et al.* 2008). These findings are consistent with the findings of various other studies (Esrey *et al.* 1985; Esrey *et al.* 1991; Prüss-Üstün & Corvalán 2006; Guerrant *et al.* 2008). While the evidence is more limited, Petri identifies a number of studies linking specific diarrhoeagenic pathogens to malnutrition, including pathogenic *Escherichia coli*, *Shigella*, *Giardia* and *Cryptosporidium* (Petri *et al.* 2008).

Soil-transmitted helminth infections, or helminthiasis, can be prevented with adequate sanitation (Strunz *et al.* 2014b) and are strongly associated with childhood undernutrition (Prüss-Üstün & Corvalán 2006). In particular, more severe cases of ascariasis and trichuriasis are associated with growth faltering in children (O'lorcain & Holland 2000; Hotez *et al.* 2004; Bethony *et al.* 2006). Hookworm infections during pregnancy can lead to malabsorption of nutrients and maternal anaemia, which in run are associated with stunting at birth (Black *et al.* 2013). Brooker and colleagues estimate that in sub-Saharan Africa, over a quarter of all pregnant women are infected with hookworm (Brooker *et al.* 2008).

There is growing evidence linking symptomatic and asymptomatic enteric infections to EED. This syndrome was first described in the 1960s (Cook *et al.* 1969) and referred to as 'Tropical Enteropathy' (or 'jejunitis'). The renaming to environmental enteropathy in the 1980s and 1990s (Fagundes-Neto *et al.* 1984), and more recently to EED (Keusch *et al.* 2013; Keusch *et al.* 2014), reflects a growing appreciation of the role of the environment in the development of this condition. EED is an asymptomatic syndrome causing chronic inflammation, reduced nutrient absorption of the intestine and a weakened barrier function of the small intestine (Keusch *et al.* 2014; Crane *et al.* 2015). These abnormalities in gut function and structure may have profound consequences for affected children, including deficits in growth, early childhood development and immune function (McKay *et al.* 2010; Korpe & Petri 2012; Keusch *et al.* 2014; Crane *et al.* 2015). Although

more research is needed, it has been argued that EED, and not diarrhoea, may be the primary causal mechanism linking WASH to child growth (Humphrey 2009a). One observational study in Bangladesh has shown that children living in households with improved WASH are both less likely to have EED, measured by lactulose : mannitol ratios in their urine [a measure of gut permeability (Lunn *et al.* 1991)], and are less likely to be stunted (Lin *et al.* 2013).

### *Social and economic mechanisms*

Another important relationship is the energy cost of carrying water for long distances from the source to the home. White *et al.* (1972) estimated from various sources that the average woman, carrying a typical load of 20 L on level ground, would consume some 39 cal per kilogramme of body weight per hour. With an assumption that 1 g of maize meal yields 3.5 cal, the average cost of water in East Africa, where most people required less than an hour to collect water, was estimated as US$25 per year.

When the water-carrying is performed by professional vendors, as is more often the case in urban areas, it is far more expensive to the consuming household. Typically, vendor prices are 10 to 20 times greater than the prices charged by the official water utility, amounting on average to some 20% of the household's income (Zaroff & Okun 1984). The prices may seem exorbitant, but this reflects the inefficiency of water transportation by such technologies as hand trolleys, donkey carts, jerry cans and buckets. If the vendors' prices are understandable in terms of their technology, how are we to understand the willingness to pay of the customers? Seen as a purchase of time, rather than water, the transaction is not as unfavourable as it might seem. Whittington *et al.* (1990) studied the options open to the customers of vendors in Ukunda, Kenya, and found that they usually chose the more costly, time-saving option only if the trade-off valued their time at more than the unskilled wage rate.

However, that does not per se render it economic for a poor family to opt for the water vendor over collecting water themselves because there may little or no spare income within the household budget to pay for water. The poorer the family, the less remains after food expenditure and so greater is the proportion of household expenditure devoted to food. This relationship is known as Engel's Law – not after Friedrich Engels, the co-founder of Marxist theory, but for the 19th century Saxon Government accountant Ernst Engel (1821–1896) who first observed this relationship between income and food expenditure (Houthakker 1957).

The pie chart (Fig. 2) shows the breakdown of a typical weekly budget of a household in the low-income areas around Khartoum, Sudan. It is striking that water already accounts for almost 30% of the household budget, and food two-thirds of the budget. So, imagine for a moment that this is *your* family budget, and that the water price has just doubled; it is hard to see how to meet this need for additional but essential expenditure, without taking from the food budget.

Thus, water supply affects nutritional status not only via the complex metabolic links described in the previous text and elsewhere in this series of papers, but also by the most direct route imaginable: the high cost paid for water by the poorest – and the poor pay for water at by far the highest cost – which leaves them without insufficient funds for an adequate diet. Indeed, bearing in mind the impact of nutrition on mortality, many of the poor pay for water with their very lives.

The fact that poor WASH brings a risk of death from diarrhoeal disease may help to explain why people are

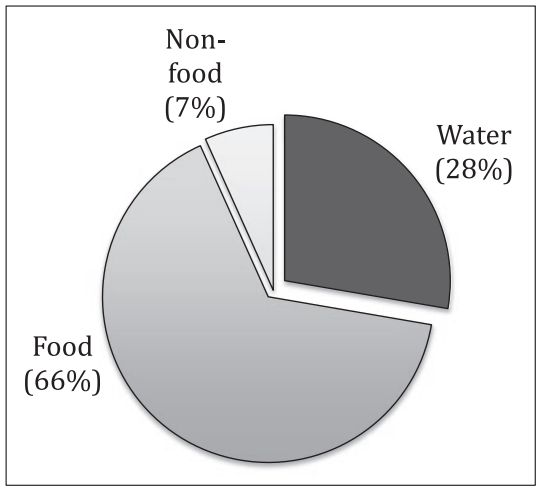


**Fig. 2.** Typical breakdown of weekly household expenditure in low-income areas of Khartoum, Sudan (1987). Source: Cairncross & Kinnear 1992.

willing to pay such a high price for water. Table 1 illustrates the inelasticity of demand for it. While the residents of Karton Kassala had to pay three times more than the people in Meiyo for their water, they used roughly the same amount of water per capita – if anything, slightly more. This lack of elasticity with regard to price was accompanied by income inelasticity of demand; households with a wide range of incomes were using roughly the same amounts of water.

These two findings have important policy implications. First, the income inelasticity means that the poorest households are paying the greatest proportion of their income for water, although they can least afford it. Second, the demand inelasticity means that price is highly sensitive to supply. Indeed, cases were found in Sudan where a slight constraint on the availability of water to fill the vendors' donkey carts led to a doubling or tripling of the price. The contrary is also true; facilitating the business of the water vendor (for example, by drilling more boreholes and offering credit to buy carts and donkeys) should lead to a substantial drop in the price of water. This drop in water prices will free up expenditure for the food budget, especially in the poorest households who most need it.

From this perspective, WASH can appear as a Holy Grail of community-based nutrition projects: delivering savings for more food, particularly to the poorest. As water is regarded as 'women's business', the savings go directly into the pocket of the housewife and mother, the member of the household who may be best placed to ensure that children benefit. The lack of studies documenting this in the literature is evidence of the difficulty of cross-sectoral vision and collaboration. Hopefully, we are now in more enlightened times, when nutritional benefits are achieved by interventions more subtle than handing out food.

**Table 1.** Inelastic demand; water prices and observed daily per capita water consumption in two low-income areas of Khartoum, Sudan, 1987

| | Meiyo ($n = 22$) | Karton Kassala ($n = 28$) |
|---|---|---|
| Mean† household size | 7.3 | 8.3 |
| Mean‡ income/head (Sudanese pounds/month) | 42 | 47 |
| Mean† water price (Sudanese pounds/drum) | 1.50 | 4.64 |
| Mean‡ water consumption (litres per capita per day) | 24.2 | 27.0 |
| Mean† % of income spent on water | 16.5 | 55.6 |

Source: Cairncross & Kinnear 1992. †Averaged by household, and‡-averaged by individual.

## Experimental evidence for the effect of WASH interventions on stunting

Although a number of studies have found a significant association between access to improved WASH and improved growth after adjusting for confounding using a range of statistical methods (Esrey *et al.* 1985; Esrey *et al.* 1991; Spears 2013; Spears *et al.* 2013), a recent Cochrane review identified only five experimental intervention studies for the effect of WASH on undernutrition. These studies spanned different WASH interventions on childhood stunting: treatment of household drinking water by solar disinfection (Du Preez *et al.* 2010; Du Preez *et al.* 2011; McGuigan *et al.* 2011), chlorination (Luby *et al.* 2006), flocculants (Luby *et al.* 2006) and the provision of soap and promotion of handwashing (Luby *et al.* 2004). Critically, though, no water supply or sanitation interventions were identified. While pooled analysis found no effect of these WASH interventions on weight-for-age z scores and weight-for-height z-scores, a small statistically significant effect was reported on height-for-age z scores [0.08 *z*-score; 95% confidence interval: 0.00, 0.16] among participants under 5 years, with a larger effect for children under 2 years of age (0.25 *z*-score; 95% confidence interval: 0.14, 0.35) in subgroup analysis.

Although no sanitation interventions were identified in this Cochrane review, five trials have subsequently published results describing the effect of sanitation interventions on stunting. Two of these studies (Hammer & Spears 2013; Pickering *et al.* 2015) reported significant effects on stunting, and three found no effect (Cameron *et al.* 2013; Clasen *et al.* 2014a; Patil *et al.* 2014). Notably, the interventions for those trials reporting no effect, two in India (Clasen *et al.* 2014a; Patil *et al.* 2014) and one in Indonesia (Cameron *et al.* 2013), had very low levels of uptake and compliance, which may explain their findings of no effect. By contrast, Pickering *et al.* report that access to sanitation increased substantially and open defecation reduced as a

result of the intervention evaluated in Mali, West Africa (2015), while the intervention evaluated by Hammer & Spears in India achieved more modest increases in sanitation access (2013). This epidemiological literature confirms what is well known by many WASH implementers that the requisite changes in behaviour are hard to initiate and even harder to sustain over time.

At least three large WASH intervention studies are currently underway that will add to this evidence base and answer important outstanding questions (Humphrey 2013; Arnold *et al.* 2013; Brown *et al.* 2015). The factorial design of the Sanitation, Hygiene, Infant Nutrition Efficacy [SHINE] (Humphrey 2013) and WASH Benefits (Arnold *et al.* 2013) trials will permit the quantification of both the independent effect of WASH interventions on stunting and the combined effect of WASH and food supplementation interventions together. All three trials include biological markers of EED to assess whether improvements in WASH can reduce EED and to what extent the effects of WASH on stunting are mediated by this subclinical condition. Lastly, the interventions assessed in these trials have novel aspects, including the SHINE trial, which specifically addresses maternal and child environmental exposures, and the MapSan trial (Brown *et al.* 2015), which, for the first time, evaluates an urban on-site sanitation intervention in high-density informal settlements.

## How much stunting might be prevented with improved WASH?

The recent Lancet Series on child and maternal undernutrition came to the somewhat sobering conclusion that if it were possible to scale-up 10 'evidence-based nutrition interventions' to almost complete coverage in the 34 countries that have 90% of stunted children, the global prevalence of stunting would be reduced by just one-fifth (Bhutta *et al.* 2013). These findings along with those of other studies (Dewey & Aduafarwuah 2008) suggest that stunting is unlikely to be eliminated without addressing the underlying determinants of undernutrition alongside deficiencies in the quantity and quality of infant and child nutritional intake. This broad category of interventions that tackle the underlying determinants is sometimes referred to as 'nutrition-sensitive' interventions and includes WASH but also things such as family planning services, maternal education and social safety nets (Black *et al.* 2013). As discussed in the previous text, WASH potentially impacts stunting through multiple and interacting biological and socio-economic mechanisms that are difficult to assess independently.

At the level of public policy, internationally and nationally, much of the interest in WASH and undernutrition boils down to a basic question: how much stunting can be prevented globally with improved WASH? Various studies have estimated the WASH-attributable disease burden over the last two decades (Clasen *et al.* 2014b), with various single or multiple infectious disease outcomes included, such as diarrhoeal diseases, helminth infections, trachoma and schistosomiasis. Of these though, we are aware of only one analysis that has included undernutrition as an outcome in their burden of disease estimate (Prüss-Üstün *et al.* 2008). This study conducted by WHO categorized the effects of WASH on undernutrition as 'direct', meaning attributable deaths resulting from protein energy malnutrition, and 'indirect', meaning attributable deaths resulting from increased susceptibility to infectious diseases as a result of undernutrition. Taken together, this study estimated that in 2004, a huge number of child deaths – approximately 860 000 – caused by malnutrition might be prevented with improved WASH.

## How can WASH interventions be mobilized to eliminate stunting?

Evidence is growing that sustained exposure to enteric pathogens in early life mediated by poor WASH conditions may have profound effects on child growth and development (Lin *et al.* 2013). In addition, there are multiple social and economic mechanisms by which poor access to WASH can increase the risk of stunting and other forms of undernutrition. In light of this, there is renewed interest in how WASH interventions might be targeted or modified to best support efforts in the nutrition sector (Humphrey 2009a). This has implications for both the nutrition and WASH sectors: for the former, reform may be needed to foster and enable greater cohesion with other complementary sectors, including WASH, and, for the latter, strategies may require modification to support broader efforts to reduce childhood undernutrition.

In countries where rapid progress has been made in recent years, such as Brazil or Peru, one consistent feature has been strong inter-sectoralism (Dangour *et al.* 2013a). While such inter-sectoralism is commonly associated with success, fostering such coordination and integration under the MDG has been challenging (Waage *et al.* 2010). Under the Sustainable Development Goals, both the nutrition and WASH sectors have dedicated goals – to 'end hunger, achieve food security, and improve nutrition and promote sustainable agriculture' and to 'ensure availability and sustainable management of water and sanitation for all' – but dedicated efforts to realize synergies and remove barriers to integration are needed (Waage *et al.* 2015). One opportunity is the Scaling Up Nutrition (SUN) initiative that actively promotes national-level coordinated action across sectors to end malnutrition. Active in over 50 high-burden countries, and supported by global agencies, including donor governments, the United Nations and international civil society organizations, the SUN movement provides a basis for the 'alignment of actions across sectors and among stakeholders' (SUN 2015) and an entry point for the WASH sector.

The WASH sector, however, faces its own challenges in delivering effective, equitable and sustainable interventions, supported by well-conceived and resourced national policies and strategies (Bartram & Cairncross 2010). As highlighted in the sanitation trials discussed in the previous text, many WASH interventions are ineffective in mobilizing community uptake and achieving sustained changes in behaviour (Barnard *et al.* 2013). For example, promoting handwashing with soap and basic on-site sanitation may in principle represent highly cost-effective public health interventions (Jamison *et al.* 2006), but many of these interventions fail to catalyse significant or sustainable changes in behaviour (Curtis *et al.* 2011). Conversely, while demand is generally high for improved water supplies, many systems fail or perform poorly due to inadequate provision for the management and maintenance of the infrastructure, thereby preventing use where demand is strong. Reducing stunting will require strong WASH programmes that do not repeat old mistakes of supply-oriented, over-engineered solutions (Cairncross 1992) nor forget the most important lesson of all that people are unlikely to wash their hands or use sanitation facilities unless they actually want to do so (Cairncross 2003).

It is not clear that traditional WASH interventions or strategies will per se deliver or at least maximize the potential nutrition benefits. Traditionally, WASH interventions have focused on ensuring access to WASH for the general population to improve health and other development outcomes. Under the MDG water and sanitation target – 'to halve, by the year 2015, the proportion of people without sustainable access to safe drinking water and basic sanitation' – 'improved' water and sanitation were defined with minimum benchmarks of community water supply and basic household sanitation. While much progress has been made under the MDG target – with 2.6 billion gaining access to safe water and 2.1 billion gaining access to adequate sanitation (WHO & UNICEF 2015) – it is unclear whether a water pump located hundreds of metres from the household or a rudimentary latrine are sufficient to protect young children from the growth faltering that results from chronic exposure to enteric pathogens. And, improved hygiene, which can be highly efficacious in reducing diarrhoeal disease, was not included under the MDG target, perhaps because of the difficulty of measuring progress.

## Priorities for a nutrition-sensitive WASH sector

While more research will help strengthen future nutrition-sensitive WASH interventions, clear points emerge from the existing evidence base that can help guide the design of nutrition-sensitive WASH strategies. In essence, the challenge is ensuring that the right people receive the right interventions at the right time. This means ensuring that populations with a high burden of stunting are targeted before or when growth faltering occurs and with appropriate WASH interventions alongside more traditional nutrition-specific interventions. Reaching and protecting those at risk may require interventions that go beyond the scope of the traditional package of WASH interventions, such as 'improved' water and sanitation as defined under the MDG target, to ensure that young children are protected from exposure to enteric pathogens.

As both diarrhoeal disease morbidity and mortality (Walker *et al.* 2013.), and the process of stunting

(Shrimpton *et al.* 2001), are concentrated in the first 2 years of life, and this growth deficit is thereafter not recovered, attention should be given to how WASH might limit exposure during this specific window. The recent Cochrane review, discussed in the previous text, validates this focus, reporting that the effect of WASH interventions on stunting was greatest in children aged 0–24 months, in an individual participant data subgroup analysis (Dangour *et al.* 2013b).

Identifying dominant faecal–oral exposure pathways for young children when they are most vulnerable to the deleterious effects of contaminated environments is the first step in identifying those WASH interventions that are likely to be most efficacious. One recent study used structured observation of mother–child couples in Zimbabwe to assess faecal–oral exposure among young children and highlighted the risks associated with the consumption of soil – geophagia – and animal waste in peri-domestic areas (Ngure *et al.* 2013). A number of recent studies in Mali (Touré *et al.* 2011; Touré *et al.* 2013) and in Bangladesh (Islam *et al.* 2013) have also highlighted the risk to this age group posed by often highly contaminated weaning or complementary food. There has been growing concern, too, about the safe disposal of children's faeces, which are generally not disposed of safely, as they are often considered to be less pathogenic than those of adults, although the reverse may be true (Brown 2003).

WASH interventions that target critical exposure points for young children should be prioritized alongside relevant nutrition-specific priorities, such as improving infant and young child feeding (WHO & UNICEF 2003). Such WASH interventions might logically include infant food hygiene – the safe preparation, storage and reheating of infant foods – controlling or supervising exploratory play to limit exposure to contaminated soil, fomites and objects (Prendergast & Humphrey 2014) and ensuring that child faeces are disposed of safely. From the perspective of the nutrition sector, this focus and package of interventions is hardly a new concept. Building on a series of seminal studies in the 1970s that demonstrated the effect of repeated infections on growth in early childhood, Mata highlighted the importance of the 'matro environment' and the 'maternal technology', which included 'handwashing… avoidance of faeces during meal preparation and eating times, (and) adequate preservation of food)' (1979).

Mirroring a wider debate in the field of international development and global health, there has been an increased focus on equity and non-discrimination within the WASH sector. Disaggregating MDG progress data by wealth quintile reveals markedly different rates of progress between groups categorized by wealth, with the slowest progress among the poorest (UNICEF 2010). If WASH sector investments are to support efforts to reduce stunting, identifying where stunting is spatially and socially clustered and targeting these populations will be important. As poverty, undernutrition and poor infrastructure often coincide, the potential for positive synergies is high. The public health benefits of targeting WASH interventions at stunted populations are twofold: firstly, that reductions in stunting might be accelerated if WASH interventions deliberately target children at risk, and, secondly, that the impact of WASH on diarrhoea and other diseases might be enhanced by targeting undernourished children who are more susceptible to infection and related mortality (Caulfield *et al.* 2004).

## Conclusions

Improved access to safe and sustainable WASH brings a broad range of well-documented and widely recognized health and non-health benefits. In addition, current evidence suggests that WASH can also bring significant gains in tackling childhood undernutrition. Whether it is by the generally better investigated pathways of enteric pathogen exposure or the plausible but less well-investigated social and economic pathways, poor WASH access is intimately linked to childhood growth and development. Realizing the potential contribution of WASH to global efforts to end stunting will require stronger coordination but may also require that WASH programmes and interventions are modified. While WASH alone will not eliminate stunting, it does have the potential to accelerate progress on eliminating stunting as a critical component of comprehensive strategies.

## Source of funding

The time of both authors was supported in part by the SHARE Research Consortium funded by the UK Department of International Development.

## Contributions

This article was jointly conceived and written by both authors.

## Acknowledgements

We would like to thank our reviewers who, though anonymous, provided very helpful feedback on the first draft of this paper. We would also like to thank UNICEF for organising the 'Stop Stunting' Conference which provided the impetus for this paper, and, in particular, to thank Victor Aguayo and Sanjay Wijesekera from UNICEF for their support.

## Conflicts of interest

The authors declare that they have no conflicts of interest.

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# 3

## Chapter 3: Water, Sanitation and Hygiene, and Enteric Pathogen Exposure

The initial motivation for this thesis was to investigate how access to water, sanitation and hygiene (WASH) services influenced childhood undernutrition, and to design and evaluate targeted WASH interventions that might reduce undernutrition in high-burden settings. The review presented in Chapter 2 addressed these two points directly. Based on the available evidence, the conclusion of this review was that unsafe WASH conditions can have a detrimental effect on child growth and development due to the resulting sustained exposure to enteric pathogens. Whilst the long-term goal must be universal access to safely managed WASH services – as enshrined under Sustainable Development Goal (SDG) targets 6.1 and 6.2 – the review highlighted the need for targeted interventions that might reduce exposure to enteric pathogens among vulnerable groups at critical moments.

On the basis of the findings presented in Chapter 2, I decided to focus on the more proximal outcome of enteric pathogen exposure rather than outcomes related to chronic undernutrition. My decision reflected the fact that chronic undernutrition is a more distal and multifactorial outcome that can be less amenable as a primary outcome in robust epidemiologic evaluations of complex interventions such as WASH. Beyond this, growth faltering is a dynamic process occurring over years, requiring long periods of follow-up that is often not feasible during a single research study. WASH evaluations with nutrition outcomes have produced mixed results and several high-profile trials reporting null effects of multiple combinations of WASH interventions on nutrition outcomes have led to much debate about the implications for both policy and research (4-7).

For my doctoral research, I decided that assessing the effectiveness of WASH intervention on enteric pathogen exposure in vulnerable populations would provide a more direct link. In this Chapter, I supplement the earlier published literature review on WASH and stunting (Chapter 2) with an additional review of the evidence concerning the burden, consequences and relationship to WASH services of enteric pathogen detection in children. Furthermore, I discuss the implications of pathogen transmission on historical and current global targets and WASH service benchmarks.

## The burden of diarrhoeal disease related to enteric pathogen exposure

Diarrhoeal diseases remain a major global health concern. Whilst diarrhoea can be caused by non-infectious factors, such as inflammatory bowel diseases like Crohn's disease and malabsorption disorders, the vast majority of cases result from exposure to infectious agents(8). These infectious agents encompass a wide range of bacteria, viruses and protozoa. The transmission of these pathogens between infected and susceptible individuals occurs via multiple environmental pathways famously described by Wagner and Lanoix (1958) in their "f-diagram". The relative importance of these different diarrhoeagenic pathogens varies significantly by setting and population (9). Two major studies in the last decade sought to strengthen our understanding of the etiology of diarrhoea in high-burden settings. The multi-site Global Enteric Multi-Centre study (GEMS) to identify the etiological agents for moderate and severe diarrhoea (MSD) in seven high-burden countries in Africa and Asia. The GEMS study reported that the four leading causes of MSD were rotavirus, *Cryptosporidium*, *Escherichia coli* producing heat-stable toxin (ST-ETEC; with or without co-expression of heat-labile enterotoxin), and *Shigella* (9). And the Etiology, Risk Factors, and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development Project (MAL-ED) study also sought to identify the etiological factors for diarrhoea in eight different high-burden settings across Africa, Asia and Latin America (10). The MAL-ED study reported *Campylobacter,* norovirus GII, rotavirus, astrovirus, and *Shigella* as the most important causes of diarrhoea in young children (10). Both studies found substantial heterogeneity in the aetiology of diarrhoea across the different sites and marked differences between age-groups.

The importance of WASH in reducing the burden of diarrhoeal disease is that these interventions act as barriers to faecal-oral transmission. Whether sanitation as a primary barrier which prevents the release of human faeces directly into the environment, or water interventions that either remove or deactivate water borne pathogen or improve access to enable domestic hygiene practices, or handwashing with soap, these all act to prevent transmission via the pathways of water, food, flies,

soil and surfaces, and hands or person-to-person contact (11). A systematic review for the effectiveness of water, sanitation and hygiene interventions on childhood interventions identified 124 relevant interventions. In the meta-analysis, they estimated that these interventions reduced the risk of diarrhoea by between 30-50% (12). A separate burden of disease analysis estimated that meeting the Sustainable Development Goal (SDG) targets (6.1 and 6.2) for water, sanitation and hygiene would prevent almost 70% of the global burden of diarrhoeal disease and, as a consequence of prevented diarrhoea, 10% of the global burden of undernutrition (13).

The global burden of diarrhoeal disease has reduced markedly since the 1980s. Several factors have contributed to this including the advent of the simple low-cost intervention of oral rehydration therapy (ORT), increased access to health services combined with improved treatment protocols for acute diarrhoea, and the inclusion of rotavirus vaccination within nation al routine immunization programmes in high burden settings. In addition, access to safe water and sanitation services has steadlily increased in all regions of the world, with notable decreases in the number of people reliant on surface water for drinking water and open defecation in lieu of sanitation facilities. Despite this progress, the burden of disease remains high. Much of the reduction in diarrhoeal disease attributable disability – a combined measure of morbidity and mortality - commonly expressed in disability-adjusted life years (DALYs) – reflect a large reduction in mortality but much more limited reduction in morbidity.  Despite this, the burden of disease remains high. Estimates of the year 2021 were that diarrhoeal diseases caused 1.17 million deaths globally and 59 million DALYs with children accounting for approximately a third and a half of these deaths and DALYs respectively.

### Molecular multiplex methods for stool-based enteric pathogen detection

Molecular methods have transformed many scientific fields, including epidemiology and infectious disease microbiology. The advent of stool-based multiplex PCR for pathogen detection offers highly sensitive methods for the simultaneous detection of

multiple pathogen associated gene targets. These methods are of particular interest for diarrhoeal diseases given the diverse aetiologies across settings and populations and the propensity for multiple and/or asymptomatic enteric infections (14). Pathogen detection by these methods is predominantly asymptomatic, meaning the individual testing positive does not have diarrhoea. Conversely, co-detections are common in those with diarrhoeal symptoms so – without an appropriate study design - it is difficult to then attribute those symptoms to one of the pathogens detected. As such, detection of a pathogen does not alone confirm disease nor the etiology of symptoms. However, detection does provide a robust measure of prior exposure to the pathogen or pathogens detected. Far more than in clinical epidemiology which is useful in environmental health where the objective is to prevent exposure versus diagnosing and treating an infection after exposure has occurred.

A study by Amar and colleagues (15), in the United Kingdom, provides an illustrative example of the value of these new methods. PCR assays were used on over 4,000 archived stool samples from the Infectious Intestinal Disease (IID) case-control study (1993-1996) (16). In the original IID study, using conventional microbiology, pathogens were identified in only 49% of diarrhoea cases and 19% of controls. Using multiplex PCR methods, though Amar and colleagues identified pathogens in 75% of diarrhoea cases and 42% of controls. In addition, in 41% of cases and 13% of controls multiple pathogens were detected. These findings have been mirrored in several studies in high-burden settings for diarrhoeagenic enteric pathogens (17-21).

Whilst these methods offer ever more rapid and more sensitive measures for enteropathogen detection in environmental and biological samples, there are important limitations. Whilst their laboratory performance is high, the clinical performance can be poor with a high risk of false positives due to detection of naked nucleic acids, laboratory contamination, and, most importantly, non-viable organisms (22). Particularly in high burden settings, where there is often a high prevalence of asymptomatic infections and sustained shedding post-infection, there is a risk of detecting DNA without or after disease (23, 24). In this light, these molecular methods may be seen as highly sensitive direct measures of exposure, rather than infection,

providing new insights into enteropathogen transmission and carriage. This information is useful both as a measure of the effectiveness of WASH interventions in preventing exposure and as an outcome in trials which lies on the causal pathway to disease, and other important consequences, as discussed below.

### Enteric pathogen detection in WASH studies

Trials of WASH interventions have overwhelmingly relied on participant reported diarrhoea, which is inherently subjective and vulnerable to reporting bias. This risk of bias is further compounded by WASH trials being almost exclusively open label as they cannot be blinded. A few examples exist of blinded trials of point-of-use drinking water technologies (25) but the ethical challenges of blinding in WASH trials and objective risk to participants have been demonstrated (26). The combination of subjective outcome measures with open label trial design make most WASH trials focused on diarrhoea at significant risk of bias (27). These limitations have been discussed extensively, particularly for point-of-use water treatment interventions (28, 29).

Stool-based detection of enteric pathogens offers a powerful alternative to diarrhoea as both an objective measure of exposure to these pathogens and as an indicator for health risk as the detection of pathogens in stool lies on the causal pathway to diarrhoeal diseases and related consequences such as malnutrition (30). Beyond this, the ability to simultaneously detect a wide range of enteric pathogens provides additional information about the enteric diseases in circulation within a given population that can inform public health strategies (31). Stool-based pathogen detection has been used as an outcome in several WASH-related studies. These include observational studies to understand environmental risk factors for exposure to enteric pathogens (32-34) and in intervention studies to evaluate the effectiveness of WASH intervention in preventing exposure to enteric pathogens (35-37).

Multiplex methods have also been used to simultaneously detect a range of enteric pathogens in environmental media relevant to WASH (38). There are a number of recent

studies that have used PCR methods to measure enteropathogens in relation to faecal-oral transmission routes, including in drinking water (39-41), in food (42-44), in soil (45, 46), on fomites (46), on hands (47, 48) and on or in flies (49). A recent (2024) systematic review and individual participant data analysis pooled data from nine studies on enteric pathogen detection in the environment and in children and demonstrated that detection in the environment was associated with subsequent detection in child stool, thereby demonstrating the causal chain from exposure to infection (50).

### Consequences of enteric pathogen exposure

There are multiple interacting consequences of enteric pathogen exposure in childhood beyond diarrhoeal disease. Here, I briefly describe four of these: undernutrition, environmental enteric dysfunction, chronic inflammation, and oral vaccine blunting. Critically these relate to the effects of repeated or chronic exposure to enteric pathogens in early life and these consequences – such as stunting - bring longer run risks which persist through later childhood and adult life.

There is a large literature dating back decades on the association between diarrhoea and chronic undernutrition or stunting in children. An influential WHO monograph published in 1968 (51) described this relationship, emphasising its bidirectional or circular nature whereby diarrhoea leads to malnutrition and malnutrition in turn renders the child more susceptible to subsequent infection. Research over subsequent decades and across different countries and regions has demonstrated robust associations between the number of diarrhoeal episodes experienced and growth faltering and risk of stunting (52-56). At the same time, studies confirmed the bidirectional nature of this relationship as described by Scrimshaw and colleagues (51) by showing that malnutrition, and specifically growth faltering, acts as risk factor for increased frequency and duration of diarrhoeal diseases (57) as well as the severity of episodes and risk of mortality (58)

Whilst diarrhoea, irrespective of the aetiology, has been shown to be strongly correlated with growth faltering (52, 59), stool-based asymptomatic pathogen detection is also associated with malnutrition. Whilst diarrhoea and enteric pathogen detection operate on the same pathways – ie diarrhoea disease requires prior exposure to and infection by diarrhoeagenic pathogens - stool-based enteric pathogen detection is predominantly asymptomatic. And, asymptomatic detection of various different enteric pathogens have been found to be associated with childhood malnutrition (60). A longitudinal multi-site study found that diarrhoeal episodes were associated with only small decreases in growth but asymptomatic detection of different enteric pathogens was associated with much larger decreases in growth by two years of age(61). In their analysis, the authors estimated that preventing exposure to just four pathogens – *Shigella*, enteroaggregative *E coli*, *Campylobacter*, and *Giardia* - in the first two years of life could increase the mean length-for-age z-score at two years of age by 0.24 (95% CI 0·08 to 0·41; 0·75 cm) equivalent to between 0.4 – 1.2 cm in height gains across their study sites(61).

Chronic enteric pathogen exposure has been linked to the onset and severity of "environmental enteric dysfunction" (EED) – a sub-clinical syndrome of the lower intestine. This syndrome was first described in the 1960s (62) and referred to as 'Tropical Enteropathy' (or 'jejunitis') then Environmental Enteropathy in the 1980s and 90s (63) and more recently to EED (64, 65), reflecting a growing appreciation of the importance of environmental exposures. EED is a generally asymptomatic condition characterised by chronic inflammation, blunting of the gut villi leading to reduced nutrient absorption of the intestine, and a weakened barrier function of the small intestine (64, 66). These abnormalities in gut function and structure have profound consequences for affected children, and are strongly associated with growth faltering (67), early childhood development and immune function (64, 66, 68, 69). The MAL-ED study found that the robust association between enteric pathogen exposure in early childhood and subsequent growth faltering was mediated most clearly through one component of the EED syndrome, systemic inflammation (70). Whilst other potential contributing determinants have been suggested (71), improving access to WASH services has been proposed as preventive measure (72, 73). One observational study in

Bangladesh has shown that children living in households with improved WASH are both less likely to have EED and less likely to be stunted (74) but intervention studies of WASH interventions are less conclusive (37, 75, 76).

### Access to WASH services and the transition from the MDGs to the SDGs

Chapter 2 was completed early on in the Sustainable Development Goal (SDGs) period (2015 – 2030) and focused on the progress that had been made during the Millennium Development Goals (MDGs) period (2000-2015). Here I supplement that earlier review to discuss the changes in normative standards for WASH services and the more ambitious levels of service under the SDG. These changes have important implications for how we approach research to understand the effectiveness of WASH interventions in preventing enteric pathogen exposure.

The MDGs included a water and sanitation target to reduce the proportion of people without access to safe drinking water and basic sanitation. Safe drinking water required access to an improved drinking water source which is protected from external contamination, including both human and animal waste (77). And basic sanitation required private access, ie not shared by more than one household, to a sanitation facility that hygienically separates human excreta for with human contact (77). Several limitations of the MDG target were important in the design of the SDG targets for water, sanitation and hygiene. These included the following five points. First the MDG target was proportional and not universal as it called for a halving of the proportion without access rather than ensuring a minimum level of access for all. Second, whilst the normative ambition for sanitation access was a facility at the household-level (by extension of not being shared with other households), for water access it could be a shared water source and distant to the household. Third, for sanitation specifically, the normative standard of an improved facility did not consider the full sanitation chain, specifically if waste contained in facilities was then safely transported, treated and disposed. Four, for water, there was no consideration of continuity, ie whether water supply was vulnerable to interruption, the actual microbial and chemical quality of the

drinking water which is often compromised even in nominally "protected" sources. And, lastly, basic hygiene – that is washing hands with soap and water – was not included in the MDG target. These five limitations have shaped the normative ambition of SDG 6, targets 6.1 and 6.2.

The SDGs were agreed in 2015 with a broad set of goals for sustainable development to be achieved by 2030. Whilst under the MDGs, water and sanitation were a target only within a broader goal of ensuring environmental sustainability, under the SDGs there was a dedicated goal to, "Ensure availability and sustainable management of water and sanitation for all", and within that included one target specifically on drinking water (6.1) and one on sanitation and hygiene (6.2). The goal was for *universal* access to WASH services and the level of ambition for water and sanitation was for "safely managed services". A safely managed water service is defined as an improved water source that is available on premises/at the household, available when needed, in other words a continuous and sufficient supply, and free from microbial contamination (no detectable E. coli per 100 mL) and priority chemicals (notably arsenic and fluoride) (78). A safely managed sanitation requires access to an improved facility at the household-level but the waste must be disposed of safely which can be that it is safely contained in situ, stored temporarily and then transported to and treated off-site, or safely transported as wastewater via a sewered system to a centralised treatment facility. Lastly, basic hygiene – defined as a household handwashing facility with soap and water available – was included alongside safely managed water and sanitation and services. In sum, the SDGs represented a step change in normative ambition with a global goal of universal access to safely managed water and sanitation services combined with basic hygiene facilities.

Despite this higher level of ambition enshrined in the SDG, the goal of universal access to safely managed WASH services will not be met. To do so would require a fivefold increase in current progress - or in absolute terms providing access to these services to over two billion people in the next four years - which cannot realistically be achieved. Under the SDG, the WHO/UNICEF Joint Monitoring Programme (JMP) – the UN body responsible for monitoring progress against SDG targets 6.1 and 6.2 – have adopted a

ladder approach under which water, sanitation and hygiene access is conceptualised as “service ladders” with the rungs running from the lowest level of service, surface water for drinking water, and open defecation for sanitation, to the highest, safely managed water and sanitation. Access to basic WASH services has also increased with 91% and 81% of the world’s population having access to at least basic water and sanitation respectively. At the same time, there has been progress increasing access to both safely managed water (from 69% to 73% of the global population) and sanitation (from 49%-57% of the global population) services (79). These two higher rungs of service – “safely managed” and “basic” - carry different costs and offer different benefits and there can be a tension as to which should be prioritised especially in resource scarce environments (80).

Access to safely managed services is a distant prospect in many high-burden settings and for many vulnerable groups. The necessary scale-up of investment to establish systems supported by the effective governance and regulation required for safely managed services may require years or decades for some populations who face immediate and severe health risks. In these settings, access to basic services is the immediate priority as an intermediary step towards safely managed (80).

### The SDG targets and enteric pathogen exposure

From an infectious disease perspective, the risk reduction from safely managed services is generally much greater than for basic services. For diarrhoeal diseases, which account for the majority of WASH related burden of disease, a systematic review and meta-analysis confirms the marginal benefits of safely managed services (12). Comparing an unimproved drinking water source to a basic level of service reduces the risk of diarrhoea by approximately 20% (relative risk [RR]: 0.81, 95%CIs: 0.70-0.94) compared to approx. 50% reduction for a safely managed water service (RR:0.48, 95%CIs: 0.26-0.87). And, basic sanitation, compared to unimproved sanitation, reduced the risk of diarrhoea by 20% (RR:0.79, 95%CIs: 0.61-1.03) compared to a near 50% reduction for safely managed sanitation (RR:0.79, 95%CIs: 0.61-1.03).

In terms of the relative difference in enteric pathogen exposure associated with these different levels of service there is a very limited literature. Studies have reported a high prevalence of enteric pathogen detection (90-99% positive for one or more pathogen) among children in areas with predominantly unimproved or basic services, including in Mozambique (34), South Africa (81), Kenya (82), Bangladesh (35). There are very few studies which have estimated the detection prevalence in settings with at least safely managed WASH services but one study in Sweden reported a detection prevalence of <1% among young children (83) demonstrating the huge difference between settings. Of the few intervention studies that have assessed the effectiveness of WASH interventions on stool-based enteric pathogen detection, all have focused on basic or limited WASH interventions. None of these trials found the WASH interventions reduced the overall prevalence (i.e. one or more detections) of the pathogens assessed (35, 37, 81). One study in Bangladesh found that sanitation and handwashing interventions reduced *Giardia* detection but not other protozoan parasites among children at 30 months of age (84); and another study, also in Bangladesh, reported that a combined WASH intervention reduced the detection of certain enteric viruses but not bacteria or protozoan parasites (35).

## Conclusions

Drawing on the literature reviewed in this chapter and the preceding one (Chapter 2) there are several conclusions that have informed the aim and objectives for the thesis. First, there is clear evidence that sustained exposure to enteric pathogens in early childhood is associated with both symptomatic diarrhoea but also far-reaching growth and development consequences that include systemic inflammation, altered gut structure and function, and growth faltering. More specifically, a number of studies suggest that the association between asymptomatic enteric pathogen detection and growth faltering maybe independent of diarrhoea and more important. Second, the literature suggests that limited or basic WASH services as per the SDG nomenclature are insufficient to prevent this exposure and thereby mitigate its effects on child growth and development. There have been no rigorous trials to date to assess the effect of

safely managed services on enteric pathogen exposure with stool-based detection using multiplex molecular methods. However, observational evidence from high-income settings combined with the null effects from trials of basic interventions suggest that safely managed services are needed to significantly reduce chronic enteric pathogen exposure. Third, there are settings where universal access to safely managed services is a distant prospect but where there is high enteric pathogen exposure that presents significant risks for vulnerable children. Informal urban settlements combining poor WASH services and high population density, and rural areas with poor WASH services combined with food insecurity and often distant health services are two such examples. And four, there have been no studies to assess the effectiveness of targeted interventions that might reduce enteric pathogen exposure among these vulnerable groups at critical times, in the absence of safely managed services.

The aim of the thesis is therefore to evaluate the effect of two such interventions specifically designed to reduce exposure in two high-risk settings during two critical moments, weaning and treatment for severe acute malnutrition.

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# METHODS

# 4

## Chapter 4: Rationale, Design and Methods for the Safe Start trial

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| **Thesis Title** | The effect of water, sanitation and hygiene conditions on enteric pathogen exposure and related health outcomes in vulnerable children – evidence from two trials | | |
| **Primary Supervisor** | Prof. Tanya Marchant | | |

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STUDY PROTOCOL Open Access

# The Safe Start trial to assess the effect of an infant hygiene intervention on enteric infections and diarrhoea in low-income informal neighbourhoods of Kisumu, Kenya: a study protocol for a cluster randomized controlled trial

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## Abstract

**Background:** *Symptomatic and asymptomatic enteric infections* in early childhood are associated with negative effects on childhood growth and development, especially in low and middle-income countries, and food may be an important transmission route. Although basic food hygiene practices might reduce exposure to faecal pathogens and resulting infections, there have been few rigorous interventions studies to assess this, and no studies in low income urban settings where risks are plausibly very high. The aim of this study is to evaluate the impact of a novel infant food hygiene intervention on infant enteric infections and diarrhoea in peri-urban settlements of Kisumu, Kenya.

**Methods:** This is a cluster randomized control trial with 50 clusters, representing the catchment areas of Community Health Volunteers (CHVs), randomly assigned to intervention or control, and a total of 750 infants recruited on a rolling basis at 22 weeks of age and then followed for 15 weeks. The intervention targeted four key caregiver behaviours related to food hygiene: 1) hand washing with soap before infant food preparation and feeding; 2) bringing all infant food to the boil before feeding, including when reheating or reserving; 3) storing all infant food in sealed containers; and, 4) using only specific utensils for infant feeding which are kept separate and clean.

**Results:** The primary outcome of interest is the prevalence of one or more of 23 pre-specified enteric infections, determined using quantitative real-time polymerase chain reaction for enteric pathogen gene targets. In addition, infant food samples were collected at 33 weeks, and faecal indicator bacteria (*Enterococcus*) isolated and enumerated to assess the impact of the intervention on infant food contamination.

(Continued on next page)

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(Continued from previous page)
**Conclusion:** To our knowledge this is the first randomized controlled trial to assess the effect of an infant food hygiene intervention on enteric infections in a high burden, low income urban setting. Our trial responds to growing evidence that food may be a key pathway for early childhood enteric infection and disease and that basic food hygiene behaviours may be able to mitigate these risks. The Safe Start trial seeks to provide new evidence as to whether a locally appropriate infant food hygiene intervention delivered through the local health extension system can improve the health of young children.

**Trial registration:** The trial was registered at clinicaltrial.gov on March 16th 2018 before enrolment of any participants (https://clinicaltrials.gov/ct2/show/NCT03468114).

**Keywords:** Enteric infections, Diarrhoea, Child food, Infant food, Hygiene, Kenya, Kisumu

## Background

Diarrhoeal disease, a key symptom of gastro-intestinal or enteric infection, is the fourth leading cause of disability globally [1] and the leading cause of child death in sub-Saharan Africa [2]. Furthermore, there is growing evidence of the impact of sub-clinical childhood enteric infection and disease on growth and development [3, 4].

Food is likely to be an important source of exposure to enteric pathogens in early childhood. Recent studies have shown that food given to children in early childhood can be highly contaminated with faecal indicator bacteria [5] as well as specific diarrhoeagenic enteric pathogens [6]. Environmental interventions to reduce exposure to these pathogens and reduce diarrhoea have traditionally focused on improving the quality and distribution of drinking water, the management of excreta through sanitation systems and the promotion of handwashing with soap at critical times [7] but generally not on food hygiene related behaviours and infrastructure.

More than half of the world's population now reside in urban areas and over one third of this population live in 'slums or informal settlements' [8]. Although access to safe water and sanitation is generally higher in urban areas [9], the risk of enteric infection may be greatest in poor urban areas due to the combination of high population density and limited public health infrastructure [10–13]. These conditions pose multiple risks for contamination of food as supported by a recent study of pathogen diversity in infant food in low-income informal neighbourhoods of Kisumu, Kenya [6]. The 'Safe Start' trial is designed to assess whether a locally appropriate, low-cost food hygiene intervention, delivered within the context of the existing health extension system in peri-urban neighbourhoods of Kisumu, Kenya can reduce early childhood exposure to enteric pathogens.

## Methods

### Research aim and objectives

The purpose of this study is to examine the effect of an infant food hygiene behaviour change intervention on child health. The study will assess the impact of the intervention on: (1) infant health as determined by prevalence of gastro-intestinal infection and diarrhoeal; (2) specific food hygiene practices; and (3) infant food contamination.

### Study design

Our study was a cluster randomized controlled trial (cRCT) design. Clusters for the trial were defined as the catchment areas of local Community Health Volunteers (CHVs); a total of 50 CHV catchment areas were recruited into the study and randomly assigned to an intervention and control arm of the study. An overview of the study design is presented in Fig. 1 (CONSORT [14] diagram).

The primary outcome for the study is the prevalence of enteric infection at age 37 weeks (+/− 1 week). We define the prevalence of enteric infection as the presence of 1 or more enteric pathogens in child stools based on the detection of 23 genetic markers of specific common enteric bacteria, viruses and protozoan (Table 1). The secondary outcome is diarrhoea; defined as the number of days a child has diarrhoea between 22 and 37 weeks of age (+/− 1 week). Tertiary outcomes include child mortality, defined as any infant death occurring between 22 and 37 weeks of age (+/− 1 week). In addition, the study will assess the effectiveness of the intervention by measuring changes in specific food practices and in bacterial contamination of infant food.

### Study setting

The study is being conducted in two informal neighbourhoods of Kisumu, Kenya: Nyalenda A and Nyalenda B (Fig. 2). Kisumu is the third largest city in Kenya and is located in Kisumu County, on the shores of Lake Victoria, and has a population of approximately 400,000. The city is surrounded by a series of peri-urban areas sometimes referred to as the 'slum belt' [15]. These peri-urban areas have emerged due to economic migration and a lack of affordable housing [16]. Some sources

**Table 1** – Specific enteric pathogen primers and probes for TaqMan Array Card used to determine the primary outcome

| PATHOGEN | GENE TARGET | FORWARD PRIMER | REVERSE PRIMER | PROBE SEQUENCE | Ref |
|---|---|---|---|---|---|
| BACTERIA | | | | | |
| Aeromonas | Aerolysin | TYCGYTACCAGTGGGACAAG | CCRGCAAACTGGCTCTCG | CAGTTCCAGTCCCACCACTT | [2] |
| *Campylobacter jejuni/ C. coli* | *cadF* | CTGCTAAACCATAGAAATAAAA TTTCTCAC | CTTTGAAGGTAATTTAGATATG GATAATCG | CATTTTGACGATTTTTGGCTTGA | [2] |
| *Clostridium difficile* | *tcdB* | GGTATTACCTAATGCTCCAAATAG | TTTGTGCCATCATTTTCTAAGC | CCTGGTGTCCATCCTGTTTC | [2] |
| Enteroaggregative *Escherichia coli* (EAEC) | *aaiC* | ATTGTCCTCAGGCATTTCAC | ACGACACCCCTGATAAACAA | TAGTGCATACTCATCATTTAAG | [2] |
| Enteroaggregative *Escherichia coli* (EAEC) | *aatA* | CTGGCGAAAGACTGTATCAT | TTTTGCTTCATAAGCCGATAGA | TGGTTCTCATCTATTACAGACA GC | [2] |
| Enterohemorrhagic *E. coli* (EHEC) 0157 | *rdbE* | TTTCACACTTATTGGATGGTCTCAA | CGATGAGTTTATCTGCAAGGTGAT | CTCTCTTTCCTCTGCGGTCCT | [1] |
| Enteropathogenic | *eae* | CATTGATCAGGATTTTTCTGGTGATA | CTCATGCGGAAATAGCCGTTA | ATACTGGCGAGACTATTTCAA | [2] |
| *E. coli (*EPEC) | | | | | |
| Enteropathogenic | *bfpA* | TGGTGCTTGCGCTTGCT | CGTTGCGCTCATTACTTCTG | CAGTCTGCGTCTGATTCCAA | [2] |
| *E. coli (*EPEC) | | | | | |
| Enterotoxigenic | ETEC LT | TTCCCACCGGATCACCAA | CAACCTTGTGGTGCATGATGA | CTTGGAGAGAAGAACCCT | [2] |
| *E. coli* (ETEC) LT toxin | | | | | |
| Enterotoxigenic | STh STp | GCTAAACCAGYAGRGTCTTCAA AATGAATCACTTGACTCTTCAAAA | CCCGGTACARGCAGGATTACAA CATGAATCACTTGACTCTTCAAAA | TGGTCCTGAAAGCATGAATGAA CAACACATTTTACTGCT | [2] |
| *E. coli* (ETEC) ST toxin | | | | | |
| *Salmonella enteritidis* | *ttr* | CTCACCAGGAGATTACAACATGG | AGCTCAGACCAAAAGTGACCATC | CACCGACGGCGAGACCGACTTT | [2] |
| *Shigella* spp. | *virG* | TCAGAAAGGTAATTGGCATGGA | AGAACCGCGCCCAAAGA | AGGGCGGAATATT | [1] |
| *Vibrio cholerae* | *hlyA* | ATCGTCAGTTTGGAGCCAGT | TCGATGCGTTAAACACGAAG | ACCGATGCGATTGCCCAA | [2] |
| PROCESS CONTROL | | | | | |
| MS2 | MS2g1 | TGGCACTACCCCTCTCCGTATTCAC | GTACGGGCGACCCCACGATGAC | CACATCGATAGATCAAGGTGCC TACAAGC | [2] |
| VIRUS | | | | | |
| Adenovirus 40–41 | Fiber Gene | AACTTTCTCTCTTAATAGACGCC | AGGGGGCTAGAAAACAAAA | CTGACACGGGCACTCT | [2] |
| Adenovirus broad species | Hexon | GCCACGGTGGGGTTTCTAAACTT | GCCCCAGTGGTCTTACAT GCACATC | TGCACCAGACCCGGGCTCAG | [1] |
| Norovirus GI | ORF 1–2 | CGYTGGATGCGNTTYCATGA | CTTAGACGCCATCATCATTYAC | TGGACAGGAGATCGC | [1] |
| Norovirus GII | ORF 1–2 | CARGARBCNATGTTYAGR TGGATGAG | TCGACGCCATCTTCATTCACA | TGGGAGGGCGATCGCAATCT | [2] |
| Rotavirus | NSP3 | ACCA TCTWCACRTRACCCTCTATGAG | GGTCACATAACGCCCCTATAGC | AGTTAAAAGCTAACACTGTCAAA | [2] |
| PROTOZOAN | | | | | |
| *Giardia duodenalis* Assemblage A | *triosephosphate isomerase (TPI)* | TTCCGCCGTACACCTGTC | GCGCTGCTATCCTCAACTG | ATTGCGGCAAACACGTCA | [1] |
| *Giardia duodenalis* Assemblage B | *triosephosphate isomerase (TPI)* | GATGAACGCAAGGCCAATAA | CTTTGATTCTCCAATCTCCTTCTT | AATATTGCTCAGCTCGAGGC | [1] |
| *Cryptosporidium spp.* | 18 s rRNA | GGGTTGTATTTATTAGATAAAG AACCA | AGGCCAATACCCTACCGTCT | TGACATATCATTCAAGTTTCTG AC | [2] |
| *C. hominus* | LIB13 | TCCTTGAAATGAATATTTGTGACTCG | AAATGTGGTAGTTGCGGTTGAAA | CTTACTTCGTGGCGGCGT | [1] |
| *C. parvum* | LIB13 | TCCTTGAAATGAATATTTGTGACTCG | TTAATGTGGTAGTTGCGGTTGAAC | TATCTCTTCGTAGCGGCGTA | [1] |

estimate that up to 60% of the city's population reside in these peri-urban communities [17].

The counties that previously made up the Nyanza and Western provinces have relatively high levels of infectious disease morbidity and mortality. The child mortality rate for Kisumu county is 105 deaths per 1000 live births and the prevalence of childhood stunting (below-2 SD) is approximately 25% [18]. In Kisumu

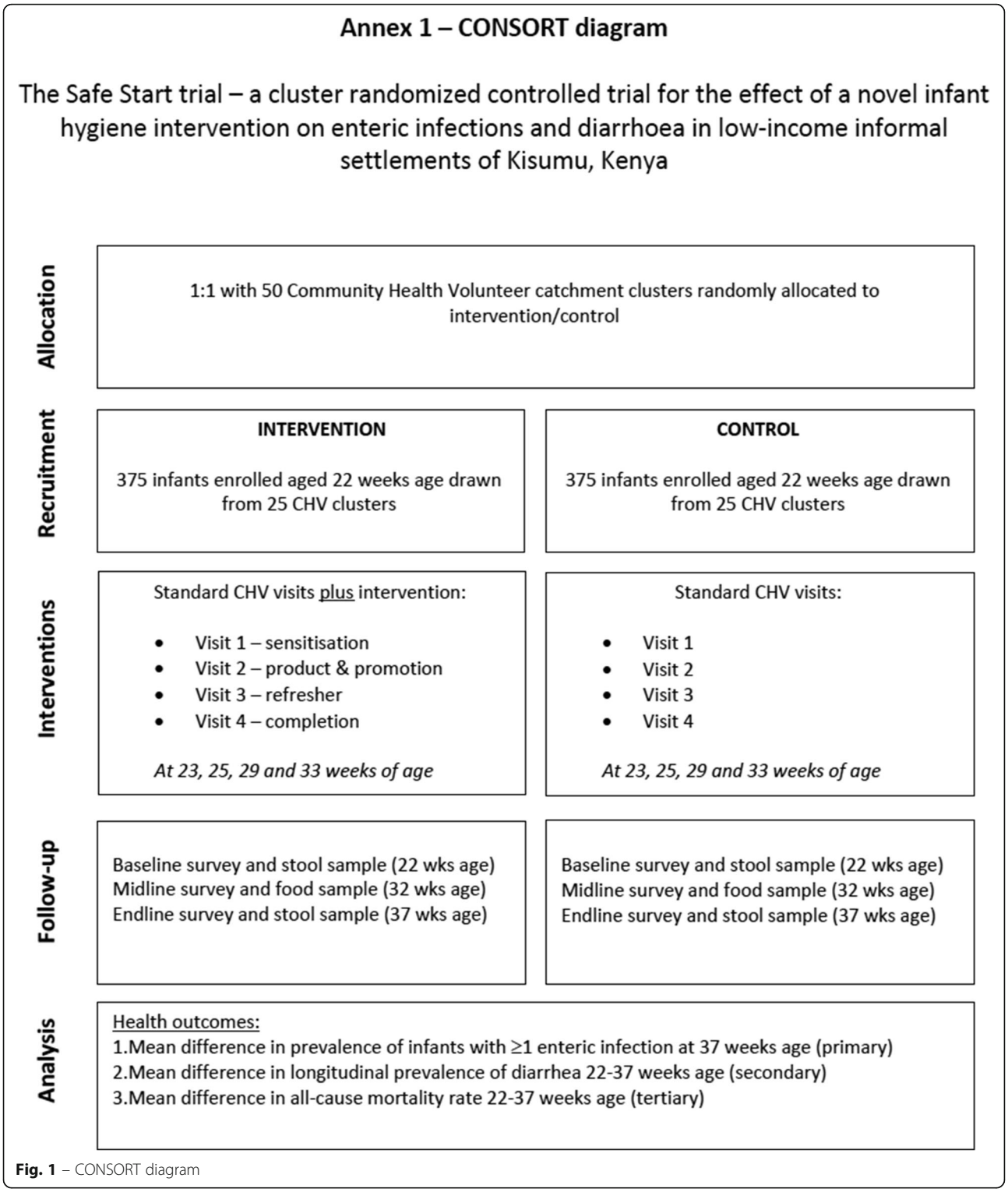


**Fig. 1** – CONSORT diagram

county, approximately 70% of all children between 12 and 23 months of age have received all recommended child disease vaccines, and it is estimated that 30% of children experiencing diarrhoea receive timely oral rehydration therapy [ORT] [18]. Two-week diarrhoeal prevalence in Kisumu is 18%, higher than neighbouring areas [18]. Data from the nearby Kenyan site of the Global Enteric Multi-site Study (GEMS) [19] reported the leading identified infectious causes of diarhhoea to be Rotavirus, *Cryptosporidium*, ST-ETEC and *Shigella*.

### Community health volunteer system

Kenya has been undergoing a process of decentralization, with many areas of policy, including the health sector and the community extension services, now the responsibility of the County Government. The Community Health Committee (CHC), is the health governance structure closest to the people at the county level. Community Health Volunteers (CHVs), who serve as frontline health workers in this decentralized system, report to the community health committee through the Community Health Extension Workers (CHEW) [20].

CHVs receive basic training to diagnose and treat illnesses such as malaria, pneumonia, and diarrhoea; make referrals to health facilities; provide health education; conduct nutrition surveillance; collect vital events data; assist with immunization and provide other aspects of maternal and child health [21, 22]. More recently, CHVs have been engaged in the promotion of some hygiene-related behaviour, including community led total sanitation (CLTS) and safe household water storage and treatment [23]. Under the current study, we collaborated with CHVs to design and test an intervention to reduce infant exposure to enteric pathogens and they are involved in the delivery of the intervention.

### Study participants

Our primary participants are infants enrolled at the age of 22 weeks (+/− 1 week), who currently reside in Nyalenda A or B, and will be living there for the subsequent five months. Our secondary participants are primary or secondary caregivers who provide care to the infant during the day and who are at least 18 years of age. A primary caregiver is defined as the person who is directly responsible for the enrolled child and a secondary caregiver is defined as any other person apart from the primary caregiver who watches the child or supports the primary caregiver.

### The Safe Start intervention

#### *Development of intervention*

We followed the Behaviour Centered Design (BCD) approach to intervention development [24]. Specific qualitative and quantitative formative research studies were implemented in a similar and neighbouring area of Kisumu city. Infant faecal-oral exposure in their domestic environment was assessed using structured observation of infants and caregivers, identifying low rates of hand hygiene among caretakers and infant food as a viable route of exposure to enteric pathogens that could be mitigated by safe preparation, storage and reheating of food [25]. Caregiver attitudes and practices in this population and the emotional and environmental drivers of food hygiene behaviours were assessed through structured observation and in-depth interviews with primary and secondary caregivers [26]. Microbiological and molecular analysis of infant food samples was used to determine the prevalence and intensity of infant food contamination with specific enteric pathogens implicated in childhood diarrhoea [6]. Various known diarrhoeagenic agents, including bacteria, viruses and protozoa, were frequently detected with at least one enteric pathogen identified in 62% of infant food samples and multiple pathogens identified in 37% of infant food. A fourth study that specifically informed Safe Start intervention delivery explored CHV schedules, routines and capacity to deliver behaviour change through direct observation, interviews, and focus group discussions. This study identified a wide range of challenges, including: poor training, lack of material resources, and limited incentives to undertake additional tasks [23].

Formative research findings led to the design of two primary candidate intervention components designed to improve food hygiene behaviours in the target population. The first component consisted of hardware items introduced at the household level to facilitate improved food hygiene behaviours. The second component consisted of motivational and educational messaging designed to improve caregiver knowledge of proper food hygiene and target the specific emotional drivers of safe food hygiene identified in formative research. The feasibility and acceptability of the two intervention components – both independently and in combination– were assessed and iteratively adapted using the Trials of Improved Practice (TIPs) methodology [27]. Details of this process are described in Simiyu et al. [28].

#### *Intervention description*

The final intervention was designed to target early childhood exposure to enteric pathogens through contaminated food. The intervention targets the following four behaviours:

1. Safe hand hygiene: handwashing with soap before food preparation and before infant feeding.
2. Safe food preparation: bringing all infant food to the boil before any feeding event.
3. Safe storage of food: storing all infant food in sealed containers.
4. Safe feeding: using designated utensils for infant feeding reserved from other use.

The intervention components use two sequential and complementary aspects of the nurture motives. The first is the desire to care for and protect a child as they grow. In formative research, “happy” was seen as marker of child fitness and health. The concept of “Happy Baby” emerged as a focal point for messaging and was

incorporated into intervention materials. The second commonly articulated aspect of nurture was the desire to ensure that the child will have a successful future. This was operationalized as messages related to a "Successful Child" and focused on ensuring that the mother provides the necessary foundation for future success. In addition to messages targeting emotional drivers, the intervention also provides the necessary foundational knowledge about food hygiene, and associated risks, but framed within an emic understanding of child health and successful parenting within the communities.

The intervention is delivered in four visits (Fig. 3) in collaboration between CHVs and specifically trained field staff. Visit 1 is a preliminary sensitization visit, led by participating CHVs in the weeks before children turn six months of age. During this first visit, CHVs reiterate existing messages regarding the importance of exclusive breastfeeding until 6 months of age, appropriate weaning foods, and their introduction after six months. The CHV also introduces new topics regarding food hygiene, including: environmental contamination, the risks associated with contaminated weaning food, and the potential health consequences - diarrhoeal disease, growth impairment, and cognitive deficits. The second visit is timed to coincide with children turning 6 months (25 weeks) old and introduces the "Happy Baby" aspect of the intervention. This household visit is designed to be a fun and lively experience for participating households and is led by specifically trained field staff who are accompanied by local CHVs. During this visit, field staff deliver a number of products designed to enable and trigger improved food hygiene practices, including: a baby bowl, a baby spoon, a baby cup, a handwashing container/station, a bottle dispenser of liquid soap (with instructions for self-refill), two deep and two rectangular sealable storage containers, and a branded "Happy Baby" feeding mat. In addition, intervention households receive a "Happy Baby" customised calendar with images that reinforce target behaviours and reference newly provided materials. Caregivers are instructed to record diarrhoea episodes on calendars between visits, ensuring that caretakers interact with and see messages. Visit 3 occurs when the child is 29 weeks old. This visit, once again lead by local CHVs, reinforces messages, discusses experiences with new target behaviours, and reviews new information on food hygiene. Visit 4 occurs when the child is 32 weeks old and introduces the "Successful Child" component of the intervention. Successful child images compliment "Happy Baby" materials by including images of older children in graduation gowns and caps. The successful child stage includes a "graduation event" for the caregiver, including a "food hygiene pledge", and a forward-looking discussion about their aspirations for the infant and how to give their child a "Safe Start" in life. As an example of the materials, we include an image of the "Successful Girl" calendar given to caregivers in the intervention group (Additional file 1).

### *Data collection*

Data are collected at three points – baseline, midline, and endline – through survey questionnaire, structured observation, along with stool and food sample collection (Fig. 3). At baseline (22 weeks of age), a short survey questionnaire is administered to the infant caregiver covering general household information, WASH access, infant health and animal contacts, with key details verified against the infant's health card (e.g. date and place of birth, vaccination status). At the same time, a stool sample is collected from the infant for analysis (procedure described below). At midline (33 weeks of age) a second household visit is made with a structured observation of infant food preparation and feeding by the caregiver, and a second short questionnaire administered. Lastly, an endline visit is completed at age 37 weeks when a stool sample is collected and a third short questionnaire administered.

Intervention 'fidelity' is assessed using process evaluation methods [29] to collect qualitative and quantitative data through in-depth interviews, focus group discussions and structured questionnaires with CHVs and caregivers among a small sample of intervention and control clusters/households. At each follow-up point, any participant deaths are recorded along with the official cause of death.

All personal identifiers collected, including names and telephone numbers, will be stored separately from other, de-identified data. All data from the surveys, stool and environmental samples will be linked through a unique household code that cannot be traced back to an individual. GPS coordinates for individual households will recorded which represents identifying data that therefore requires careful protection. The GPS coordinates themselves, and the specific locations of households on maps, will not be published or presented with results of any analyses. All physical forms will be kept in a locked file cabinet in a locked office to prevent unintended release of information. All electronic data will be encrypted and stored on secured and password protected electronic databases.

### *Environmental and clinical sample collection*

A stool sample is collected for each enrolled infant at baseline (22 weeks of age) and endline (37 weeks of age), and an infant food sample collected at midline (33 weeks of age) [Fig. 3]. For infant stool, the infant's caregiver is given several unused, clean diapers and is asked to use the diapers on the child until they defecate. Once a child has defecated in a diaper, the caregiver folds the diaper so that

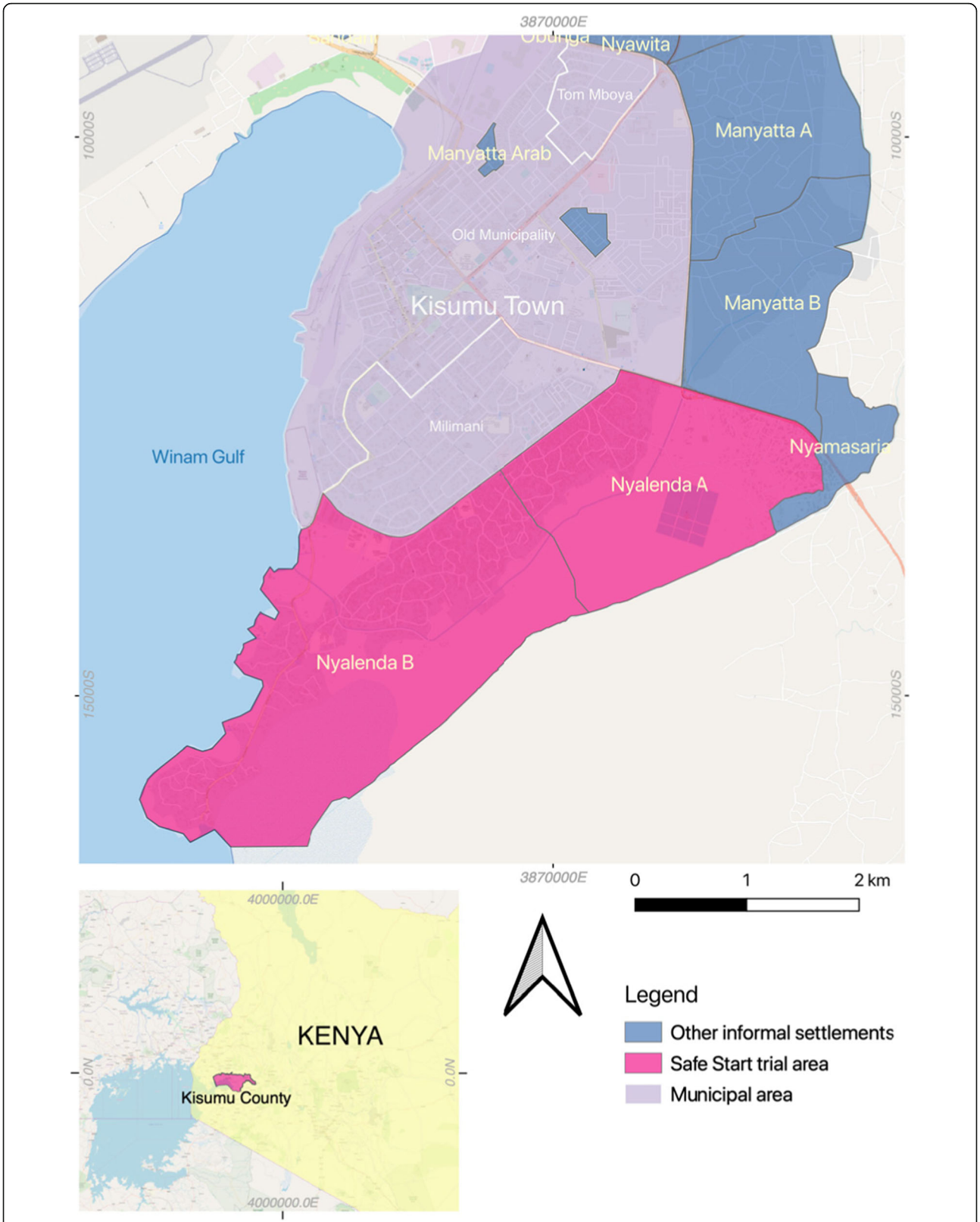


**Fig. 2** – Map showing Safe Start study areas of Nyalenda A and B (pink), two of the informal neighbourhoods around Kisumu Town in Kisumu County, Kenya

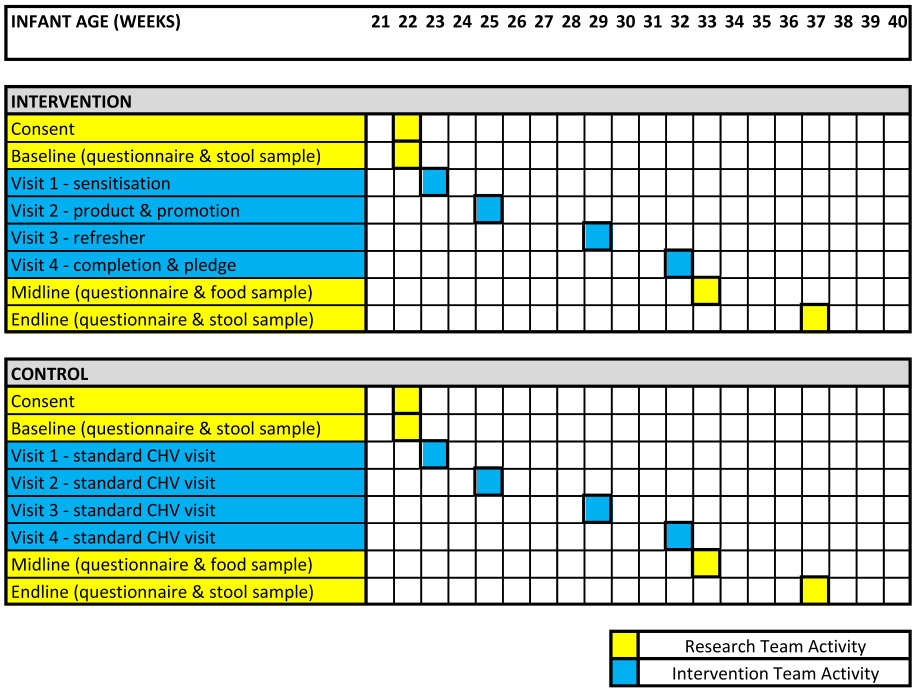


**Fig. 3** Intervention and Data Collection Schedule

the faeces is undisturbed on the interior and places the diaper in a provided biohazard bag. This procedure is used to prevent faeces samples from being collected off the ground (contaminated by soil) or from out of potties used by other children (contaminated by faeces). The bag is stored in a cool, dark, secure place until the research team returns to the household the next day and collects the sample. On the day of sample collection, the enumerator uses the scoop from the sterile collection bottle to scoop the stool from the diaper into the bottle, labelling the container with the date of collection and participant's identification number. The bottle with the stool sample is placed in a bio hazard bag and the bag placed on ice in a cooler box and transported to the laboratory. At the laboratory, a lab technician sterilizes the outside of the bio hazard bag, removes the stool collection bottle from the bag, and records the sample as received. If the infant has not defecated on the day of sample collection or the stool sample is not sufficient for collection, the enumerator informs the parent or caregiver that they will return again the next day. This continues for up to 5 consecutive days.

For the infant food sample, the research team collects a sample of food cooked during the midline observation, and again several hours later after food has been used and stored for several hours. The caregiver is asked to place a sample of food in a sterile WhirlPak bag by the same means as she would feed a child (e.g. spoon, hands). Given that levels of contamination in food may increase with time during the day, time of collection is noted. Samples are labelled (date, time and study identification number), placed immediately into a cooler box, maintained at < 10 °C with ice packs, and then transported to the laboratory for analysis.

### *Laboratory analysis*

Food samples are processed by enumerating a bacterial indicator of faecal contamination (*Enterococcus*). In brief, 1 ml (mL), 0.1 mL, and 0.01 mL dilutions of liquid foods are filtered through 0.45 μm pore-size membrane filter (Millipore Corp., Bedford, MA, USA), and the filters are cultured overnight on Slanetz &Barley Enterococcus Medium (OXOID CM0377). For solid foods, five grams are homogenized with 45 mL of sterile phosphate buffer saline (PBS), and 10 mL, 1 mL, and 0.1 mL dilutions are filtered and cultured on Enterococcus agar plates. Then the plates are incubated at 41 °C ± 0.5° for 24 h. After incubation, all light and dark red colonies are counted as *Enterococcus* and expressed as colony forming units (CFU) present per gram of food sample. A 10 ml volume of PBS used to resuspend solid food samples and wash membrane filters is processed each day as a food negative control.

A 200 mg sample of each stool sample is measured into a Zymo Shield Collection container and DNA and RNA is co-extracted using the ZymoBiomics DNA/RNA Mini kit according to the manufacture's protocol (Zymo Corp., CA, USA). DNA/RNA is immediately stored in a − 20 °C freezer until transfer to the University of Iowa for molecular analysis. A second 200 mg stool sample is

transferred to a labelled sterile Eppendorf tube and stored in a − 20 °C freezer as a repository in the event that primary samples are lost, mislabelled, or otherwise destroyed. All stools are processed in sterilized biosafety cabinets with laminar air flow, and one process negative control is prepared each day by leaving a Zymo Shield Tube open in the cabinet during stool processing, and then processing it for DNA/RNA extraction. Pathogen targets are detected and quantified by quantitative real-time polymerase chain reaction using Customized Taqman Array Cards on a ViiA7 thermocycler (Life Technologies, USA) as previously described with the exception of adding 300 uM bovine serum albumin (BSA) to reduce inhibition during PCR. Outcomes are defined as the pathogen-specific presence and concentration of individual pathogens, as well as the presence and diversity (sum of pathogen types) of all pathogens. Concentrations of individual pathogens per gram of stool are estimated by comparison of cycle thresholds of pathogen specific genes against standard curves for each reference of interest. In the event that pathogen genes are detected in process negative controls, monoplex PCR is used to verify that detection is true contamination. If negative controls are contaminated, the stool samples processed on the same day as the negative control are considered non-determined (ND) for the related pathogen.

### *Sample size calculation and analysis*

Using a standard approach for calculating sample size for cluster Randomised Controlled Trials [30] we estimated the minimum detectable difference in primary and secondary health outcome measures with a planned total sample size of 750 children (375 intervention, 375 controls) across 50 clusters (25control/25 intervention) and with an anticipated intra-class correlation coefficient (ICC) of 0.01. Our assumptions regarding baseline/control prevalence of any enteric infection and diarrhoeal disease are drawn from the most recent Multiple Indicator Cluster Survey (MICS) estimates for the prevalence of stunting and recent diarrhoea in Nyanza province [18], and the Demographic and Health Surveillance (DHS) survey national urban estimates for Kenya [31]; alongside, the national (Kenyan) and global estimates for prevalence of any enteric infection from the Global Enteric Multi-country Study (GEMS) [19]. In the absence of published effect size estimates for similar early childhood interventions on enteric infection prevalence and our assumption regarding effect size is cautiously estimated based on the effects on diarrhoea of different WASH interventions [32].

For the primary outcome, with 750 infants enrolled, and assuming a control prevalence of ≥1 of the 23 measured enteric infections of 0.7, and an intraclass correlation coefficient (ICC) of 0.01 we would have 80% power at a 5% level of significance to detect a minimum difference between arms in the prevalence of ≥1 infection of 11%. For our secondary outcome, with 750 infants, we would be able to detect a minimum difference in longitudinal prevalence of caregiver reported diarrhoea of 7% or greater, assuming a control longitudinal prevalence of diarrhoea of 15%.

The CONSORT Statement for cluster randomised controlled trials will guide the analysis and presentation of results [33]. To assess any imbalance between arms, descriptive statistics of demographic and outcome measures (where available) will be tabulated at baseline.

All analysis will be carried out on groups as randomised ('intention to treat'). All analyses will account for the nature of the distribution of the relevant outcome and results will be presented as appropriate effects sizes at 95% confidence intervals. We account for clustering by using generalised estimating equations (GEE) and adjust for baseline differences in groups by including the cluster mean of our outcome at baseline as a covariate in statistical models. For all analyses, unadjusted and adjusted results will be presented, with covariates in adjusted analyses specified a priori.

### *Randomisation*

Randomisation was undertaken remotely by the Clinical Trials Unit at the London School of Hygiene & Tropical Medicine (LSHTM). The unit of randomisation is the CHV catchment cluster, and, in discussion with the Ministry of Health for Kisumu County, the participating 50 clusters were selected from the 94 eligible clusters in the study neighbourhoods, with eligibility determined by the presence of an "active" CHV. The 50 active clusters were then randomly allocated 1:1 into two trial arms.

### *Blinding*

This is a public health intervention seeking to change specific behaviours through direct engagement with participants such that blinding of participants to their allocation was not deemed possible. Randomisation of clusters was done remotely; enumerators, principal investigator, and trial statistician were blinded to allocation. The trial statistician will conduct final analyses blind to allocation.

### *Coordinating committees*

The Trial Management Group includes representatives from each partner organisation (GLUK, Iowa University and LSHTM) chaired by the Principal Investigators (JM and OC). Modifications required to the protocol (intervention, participants, study design, analysis methods, or outcomes) during the study will be approved by the

LSHTM Research Ethics Committee prior to implementation and the new information registered on the trial registry (clinicaltrials.gov). Need and frequency of audits for trials is independent of the investigators and is determined using a risk-based approach.

### *Adverse events*

The trial is monitored for adverse events and all reported adverse events are documented and reports are compiled on a quarterly basis. The principal investigators (JM and OC) will review any reported severe adverse events to assess the level of relatedness to intervention and take appropriate action.

### Limitations

We had initially intended for the Safe Start intervention to be delivered exclusively by CHVs to demonstrate more directly the scalability of such an intervention within the existing health system structure and resource envelope. However, findings from our formative work demonstrated that such an approach would likely place undue burden on CHVs in the context of a research project. Although delivered by specialized field workers employed for the purposes of this study, our intervention is still considered to be deliverable within the CHV system and has been endorsed as such by the Ministry of Health for Kisumu County.

## Discussion

The goal of the 'Safe Start' intervention is to demonstrate that low cost, locally appropriate food hygiene interventions which target child caregivers of weaning infants can reduce foodborne exposure to enteric pathogens and the resulting infection and disease. Our intervention, informed by extensive formative research with infants, caregivers, health extension workers and discussion with the local Ministry of Health, has the potential to be scaled up if proven to be effective.

### Trial status

Protocol version number and date: Version 1, March 01, 2018.

Date recruitment began: March 26th, 2018.

Approximate date when study will be completed: November 30th, 2019.

## Supplementary information

**Supplementary information** accompanies this paper at https://doi.org/10.1186/s12879-019-4657-0.

**Additional file 1.** Intervention materials, the "Successful Girl" calendar.

### Abbreviations

CFU: Colony forming units; CHC: Community Health Committee; CHEW: Community Health Extension Worker; CHV: Community Health Volunteer; CLTS: Community Led Total Sanitation; cRCT: Cluster Randomized Control Trial; CU: Community Unit; DHS: Demographic Health Survey; GEMS: Global Enteric Multicenter Study; GLUK: Great Lakes University of Kisumu; GPS: Global Positioning System; ICC: Intraclass Correlation Coefficient; ICH-GCP: International Council for Harmonisation – Good Clinical Practice; LMIC: Low and Middle Income Countries; LSHTM: London School of Hygiene and Tropical Medicine; MICS: Multiple Indicator Cluster Survey; MSD: Moderate and Severe Diarrhoea; ORT: *Oral rehydration therapy* (*ORT*); TIPS: Trial of Improved Practice; WASH: Water Sanitation and Hygiene

### Acknowledgements

We would like to recognize the early inputs of the late Dr. Jeroen Ensink to this project as well as his wider contribution to the field of environmental health research. As a colleague, and as a friend, he is sorely missed. We are especially grateful to the County Government of Kisumu and in particular the Departments of Public Health and Community Health Strategy for their strong and sustained support for the Safe Start trial. To the CHVs in Nyalenda A, Nyalenda B and Obunga, thank you for doing an outstanding job and for the commitment and energy you bring to your work. Finally, we gratefully acknowledge the many contributions and efforts of the Safe Start Team without whom none of this would be possible. We wish to sincerely thank Julius Otieno, Mary Aligonda, Caroline Atogo, Lilian Chebichi, Winnie Alando, Edwin Atitwa, Bonphace Okoth, John Denge and Wilson Otieno - thank you for giving 'Safe Start' your very best!

### Authors' contributions

JM: Funding acquisition, study conception, protocol design, first draft of manuscript.OC: Funding acquisition, study conception, protocol design, first draft of manuscript. SS: Contributed to protocol design, contributed to manuscript writing. RD: Contributed to protocol design, contributed to manuscript writing. KKB: Contributed to protocol design, contributed to manuscript writing. JA: Contributed to protocol design, contributed to manuscript writing. EAse: Contributed to protocol design, contributed to manuscript writing. AC: Contributed to protocol design, contributed to manuscript writing. EAll: Contributed to protocol design, statistical analysis, contributed to manuscript writing. All authors read and approved the final Manuscript.

### Funding

The Safe Start trial is funded by the United Kingdom Department for International Development through the SHARE Research Consortium (www.SHAREresearch.org). The funder has not had, and will not have, any role in the design and execution of the study, the analysis of study data nor any decisions to publish results relating to the study.

### Availability of data and materials

Not applicable.

### Ethical approval and consent to participate

The investigators of the study have training in good clinical practice and research ethics in humans, and the collection of human tissue. The study received ethical approval prior to the enrolment of participants. Ethical approval for the study were obtained from Great Lakes University of Kisumu (Ref: GREC/010/248/2016) and London School of Hygiene and Tropical Medicine (Ref: 14695), and University of Iowa (Ref: 00000099). The primary study participants are infants aged < 6 months and the secondary participants are their caregivers. We deem that the infants are unable to either consent or assent to participate so consent is sought from the primary caregiver for the infant by specialized field workers. A participant information sheet (PIS) and consent statement is read in the preferred local language (Dholuo or Swahili) and a written copy of both documents provided. Consent forms are signed and dated by the caregiver. In the case of illiterate participants, an independent witness will sign and date the consent form and a fingerprint will be collected from the participant. If study participants withdraw from the study subsequent to enrolment, any data already collected and analysed will be used, unless the participant requests otherwise, but no further analysis will be done nor samples kept.

### Consent for publication

A paragraph in the PIS informs the participant that study findings will be published in scientific journals. The statement says, "At the end of study, all

data from questionnaires and samples will be analysed by researchers working with the London School of Hygiene and Tropical Medicine, Great Lakes University of Kisumu and the University of Iowa. Results of the questionnaires will be summarized anonymously and presented at community meetings convened by Great Lakes University of Kisumu which participant's will be invited to and will be able to ask questions of the research team if there is anything which the participant does not understand. The overall results of the research will be presented to Kisumu County Ministry of Health and Published in Scientific journals."

#### Competing interests

All authors declare that they have no competing interests, whether financial or non-financial.

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Received: 28 August 2019 Accepted: 22 November 2019
Published online: 19 December 2019

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## Publisher's Note

# 5

## Chapter 5: Rationale, Design and Methods for the TISA trial

## Abstract

Severe acute malnutrition (SAM) - or severe wasting and/or nutritional oedema - results from inadequate nutrient intake and recurrent illnesses and persists as an issue of global health concern. In 2022, 45 million children were affected by acute malnutrition globally of whom 14 million experienced severe acute malnutrition (SAM), and of these only one third are estimated to have received appropriate treatment (1). In its recently updated guidelines, the World Health Organisation (WHO) continues to recommend the Community-based Management of Acute Malnutrition (CMAM) model for treatment of uncomplicated SAM cases (2). The CMAM approach is regarded as a successful innovation that has reduced costs to both the health system and the individual, and improved the cost-effectiveness of treatment (3). Despite this success, recovery, referral and relapse rates for CMAM programmes vary greatly between settings, and often fall short of the global standard of 75% recovery (4). One factor which may contribute to poor recovery outcomes for children treated through CMAM is the lack of safely managed WASH services at the household level and the resulting exposure to enteric pathogens. The Traitement Intégré de la Sous-Nutrition Aiguë (TISA) trial was designed with the Ministry of Health, and NGO Action Against Hunger, and the intervention was delivered through the existing health system. The study was a cluster-randomised controlled trial (cRCT) to assess the effectiveness of integrating a drinking water treatment and hygiene promotion intervention within the standard protocol for outpatient treatment of non-complicated SAM cases in Senegal. The primary outcome of the trial was the SAM recovery rate as defined in the national protocol of Senegal, and the secondary outcomes were weight gain, rate of referral, longitudinal prevalence of diarrhoea, prevalence of enteric pathogen detection and all-cause mortality. The trial was prospectively registered at clinicaltrials.gov (Ref: NCT04667767).

## Background

Severe acute malnutrition (SAM) is a form of wasting and is defined as a very low weight-for-height z-score (WHZ), usually specified as below -3 z-scores of the median WHO growth standards (5). It is a short-term acute condition with a high case-fatality rate that, if untreated, greatly increases both the susceptibility of children to infection and the risk of mortality from infectious diseases, such as diarrhoea and pneumonia (6). In 2022, 45 million children were affected by acute malnutrition globally of whom 14 million experienced severe acute malnutrition (SAM), and of these only one third are estimated to have received appropriate treatment (1).

Historically, acute malnutrition was predominantly managed through facility-based inpatient care in hospitals and dedicated therapeutic clinics or feeding centres. However, treatment of uncomplicated SAM cases through inpatient care carries high costs to the health system and places pressure on often stretched healthcare facilities. In addition, reliance on centralised care often presents barriers to access for vulnerable high-burden populations, due to the geographic distance to facilities and related financial costs. These challenges – and resulting low levels of treatment uptake, and poor outcomes, including high mortality rate among children with SAM – led to the development and widescale adoption of a decentralised inpatient model of care for SAM. CMAM was incorporated into the WHO Guidelines for management of acute malnutrition in 2013 (7) which were recently updated (2).

Despite the success of CMAM - as a model of outpatient treatment of non-complicated SAM – there is marked variability in recovery rates. Official reports of the recovery rate of children with uncomplicated SAM treated in OTP vary widely, ranging from 32.7% for a study in Ethiopia (8) to 86.5% in Burkina Faso (9). One reason for the observed variability in recovery rates across different settings may be domestic environmental conditions, and specifically drinking water, sanitation and hygiene (WASH). The well-described interaction between infection and malnutrition (10) may limit recovery and inhibit the effectiveness of standard SAM treatment. In general, the absence of proper sanitation, good hygiene conditions, access to safe water, and adequate water storage conditions may be an important determinant of childhood malnutrition via associated diarrhoeal

disease, helminth infections, and environmental enteric dysfunction (11). WASH interventions can reduce the risk of a range of infectious diseases, including diarrhoea (12), soil-transmitted helminths (13) and acute respiratory infections (14) and therefore might protect children from infections during treatment that may limit weight gain and recovery.

*Rationale for the TISA trial*

Over the last decade, there has been renewed interest in how interventions to improve water, sanitation and hygiene conditions at the community and household levels might reduce childhood undernutrition. There has been a strong focus on the potential role of WASH means to preventing the burden of chronic undernutrition or stunting by reducing the risk of infection and disease in children (11). Whilst the association between the burden of symptomatic diarrhoeal disease (15) and also the burden of asymptomatic enteric infection (16) is well-documented, the extent to which basic WASH interventions are sufficient to reduce undernutrition is unclear.

Furthermore, numerous studies have explored the links between different WASH interventions and childhood nutrition. A Cochrane Review reported a small but measurable benefit of WASH interventions on chronic malnutrition, or stunting, (an improvement of 0.08 height-for-age [HAZ] z-score in the intervention group compared to the control group) but no evidence of an effect on acute malnutrition, or wasting (weight-for-height z-score [WHZ]).(17) However, the studies included in that review were all of low to medium methodological quality, failed to measure, or reported low, compliance, did not target children with severe acute malnutrition (SAM) in food insecure settings, and did not measure SAM recovery rates, and the interventions themselves were not targeted at children undergoing outpatient treatment for SAM(17).

A more recent (2022) systematic review for the effect of WASH interventions on acute malnutrition (18) identified only two rigorous trials (19, 20) but both reported a positive effect on recovery of children undergoing outpatient treatment for SAM. The first of these was a cluster-randomized controlled trial (cRCT) of the effectiveness of a

"Household WASH Kit" on SAM relapse at three and six months post-discharge which was carried out in Kanem, Chad (2015-2016) (19). Among children undergoing outpatient treatment for SAM, those in the intervention arm received a kit containing a sealed drinking water storage container with a plastic cup, Aquatab effervescent chlorine tablets for treatment of drinking water, bars of soap for hand washing, a leaflet with key hygiene measures to be observed during treatment, together with weekly facility-based sessions and two home-based sessions to promote safe hygiene behaviours(19). However, the evaluated kit is not scalable (too big and too complex for emergency settings). A second site-randomized controlled trial on the effectiveness of water treatment products was carried in Sindh province, Pakistan, and the preliminary results are similar to those in Chad, but the number of clusters was low and an effect on diarrhoea reduction could not be observed (20). Whilst no trials have so far been conducted in the Sahel, there has been at least one observational study conducted which suggested an association between the lack of adequate water supply and length of treatment for malnutrition (21).

The WASH and Nutrition strategy (22) promoted by various agencies including UNICEF, ECHO, Action Against Hunger in the Sahel region since 2008 has five pillars which include the provision of a WASH package, usually at the admission of the child to CMAM treatment. The purpose of the WASH package is to reduce the risk of infections during the recovery period, thereby increasing recovery rates and reducing the number of hospital transfers/referrals and deaths. Before the inception of this trial the Ministry of Health has not provided the WASH kit as an integral part of the CMAM protocol. However, UNICEF through some health facilities in Senegal, has deployed a WASH kit comprising a handwashing station, soap, bleach, a towel and Aquatabs. These are distributed to caregivers of SAM-diagnosed children admitted as uncomplicated SAM to outpatient treatment. Prior to this trial though the effectiveness of the WASH kit had not been rigorously evaluated and the Ministry of Health sought evidence for the effectiveness of such an approach as a basis for considering its integration in the national protocol. In addition, the Ministry of Health was interested in a simpler intervention for which the procurement and distribution chain would be more robust to

avoid the frequent stock-outs experienced by UNICEF in the deployment of their WASH kit.

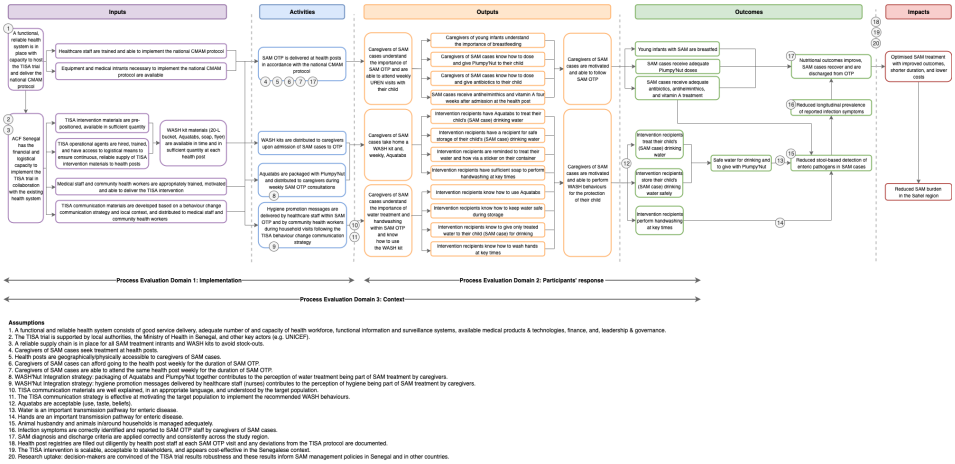
The aim of the TISA trial was therefore to evaluate the effectiveness of integrating a simplified, scalable WASH kit, including household water treatment products, a safe water storage container, and hygiene promotion, into the standard CMAM protocol and delivered through the existing structures of the health system.

## Methods

*Study design, aim and objectives*

The aim of the “Traitement Intégré de la Sous-Nutrition Aiguë” (TISA; “Integrated Treatment of Acute malnutrition") trial is to assess the effectiveness of adding a “WASH kit” to the national protocol for outpatient treatment of uncomplicated SAM cases in Senegal . The specific objectives for the trial overall were to evaluate: (1) the effect of adding the WASH kit on paediatric SAM recovery and other health outcomes; (2) the effect of adding the WASH kit on the microbial quality of stored drinking water; (3) the marginal cost-effectiveness of adding the WASH kit to the standard national protocol for outpatient treatment of SAM in Senegal; (4) the acceptability, fidelity, compliance and scaleability of the WASH kit in Senegal. In this thesis, I report results relating to the first objective and the fourth objective. A theory of change (TOC) (23) was developed to guide the design and evaluation of the strategy (Figure 1). In essence, a TOC, is, “a theory of how and why an initiative works” (24) and the TOC developed for this trial describes how hypothesized that the intervention would work. The TOC informed the design and evaluation of the intervention, the indicators which were measured from inputs through to outcomes, and made explicit the numerous assumptions.

Figure 5.1 – Theory of change for the TISA trial




Under the cRCT design, clusters representing the catchment areas for primary health care facilities (UREN) are allocated 1:1 to a control group receiving the standard outpatient treatment programme or an intervention group receiving the OTP plus a "WASH kit" (described below). Study participants are enrolled at admission to outpatient treatment, after SAM diagnosis by a nurse at the healthcare facility based on the national protocol (detailed below). After enrolment, study participants were followed up over eight weeks to assess a range of outcomes through visits to the healthcare facility and a household visit (Table 5.1). Environmental sampling of water and food is performed at the households of a sub-sample of study participants, and clinical samples are collected at the health facility at study exit (Table 5.1).

Table 5.1 - Schedule of enrolment, interventions and assessments

| | STUDY PERIOD | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Enrolment** | **Allocation** | **Participant follow-up (weeks)** | | | | | | | **Close-out** |
| **TIMEPOINT**** | **Prior** | **0** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** |
| **ENROLMENT:** | | | | | | | | | | |
| Eligibility screen | X | | | | | | | | | |
| Informed consent | X | | | | | | | | | |
| Allocation | | X | | | | | | | | |
| **INTERVENTIONS:** | | | | | | | | | | |
| Standard protocol | | ♦— | — | — | — | — | — | — | — | —♦ |
| Standard protocol *plus* WASH | | ♦— | — | — | — | — | — | — | — | —♦ |
| **ASSESSMENTS:** | | | | | | | | | | |
| Registry visits | X | X | X | X | X | X | X | X | X | X |
| Household visit | | | | | | X | | | | |
| Water sampling | | | | | | X | | | | |
| Clinical sampling | | | | | | | | | | X |

*Study Outcomes*

The primary outcome of the trial is the SAM recovery rate within eight weeks of initiating outpatient treatment for SAM. Recovery is defined as two consecutive measures at weekly facility visits with weight-for-height z-scores ≥ -1.5, if admitted based on weight-for-height z-score, or brachial perimeter (mid-upper arm circumference; MUAC) ≥ 125 mm, if admitted based on brachial perimeter, and no oedema. There are five secondary outcomes, all assessed over eight weeks subsequent to initiation of outpatient treatment for SAM. First, weight gain, defined as grams of weight gained per kilo per day between entry and exit. Second, the rate of referral, defined as the number of participants referred to the next level of clinical care. Third, the prevalence of diarrhoea, defined as diarrhoea (three or more loose or liquid stools passed within 24 hours) reported by the child's caregiver (2). Fourth, the stool-based enteric pathogen detection prevalence via a multiplex PCR assay including a broad range of bacterial, viral, protozoan and helminth targets listed below (Appendix 5).

## Study setting

The trial was conducted across four districts (Podor, Pété, Linguère, and Dahra) of two departments (Podor and Linguère) in northern Senegal with a total population of approximately 600,000 (Figure 5.2). This region has poor access to basic services (health, education, water and sanitation) and subsistence is based on agriculture or nomadic livestock practices, with traditional labor-intensive practices still prevalent. According to the projections of the *Analyse du Cadre Harmonisé* (2018), approximately 50,000 people in the departments of Linguère and Podor were at risk of food crisis during the lean season of 2019 (23).

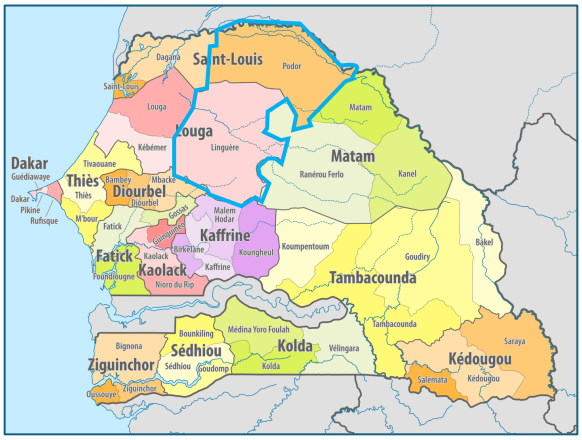



Figure 5.2 - Study area, showing the departments of Podor and Linguère

In 2023, prevalence of childhood diarrhoea in Senegal was 22% but rising to 36% among children aged 6–23 months (25), coinciding with the weaning period when children transition to solid foods and become more exposed to their environment, increasing pathogen contact. The national prevalence of SAM in 2023 was also highest in children aged 6–23 months, ranging from 1.3% - 2.4% (25).

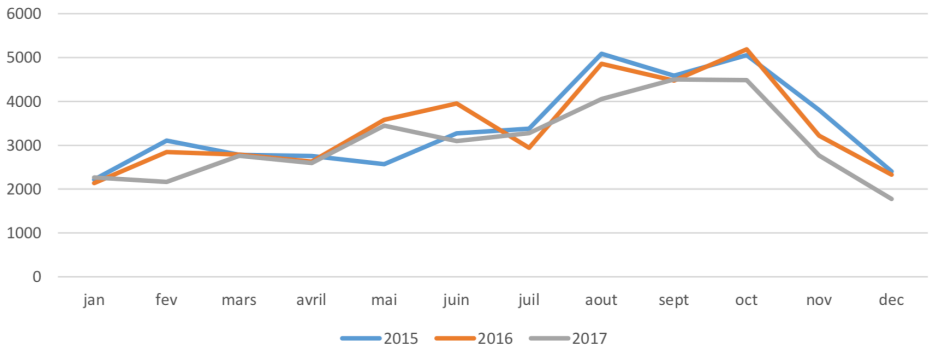



Figure 5.3 - Monthly reported admissions to outpatient treatment for severe acute malnutrition nationally

*Community Based Management of SAM in Senegal (PECMAS/ICCM+)*

Since 2013, the national protocol of the Ministry of Health for Senegal for management of acute malnutrition has recommended outpatient treatment for uncomplicated SAM cases, in line with WHO guidelines(7). Under the Prise en Charge de la Malnutrition Aïgué (PECMA) protocol uncomplicated SAM cases are diagnosed at health posts and uncomplicated cases treated as outpatients with weekly visits to the health post. The SAM prevalence is 1.1% (0.6-2.0%) in Podor and 1.8% (0.3-9.5%) in Linguere according to the the Ministry of Health's reports. In the study area there are approximately 100 health posts where the PECMA protocol is administered and in 2017 there were a total of 2,542 SAM cases reportedly admitted for treatment, of which approximately 30% abandonned treatment before discharge or referral (23).

*Participants*

All diagnosed uncomplicated SAM cases aged 6-59 months that are admitted to outpatient treatment were eligible for participation in the trial. Patients who met the diagnostic criteria for SAM were not eligible for inclusion in the trial as they should be referred for inpatient care. All eligible children for whom the parent provided written consent were enrolled. The inclusion criteria were that the child had: (1) Weight-for-height z-score <-3; and/or (2) brachial perimeter (mid-upper arm circumference [MUAC]) <115mm; and/or (3) bilateral oedema. And, the single exclusion criterion was

not being able and/or willing to participate for the duration of follow-up (8-weeks from admission).

## Study Procedures

*Randomisation, allocation and blinding*

Under the cRCT design, clusters representing the catchment areas for primary health care facilities (UREN) were allocated 1:1 to a control group receiving the standard outpatient treatment programme as per the national protocol for Senegal or an intervention group receiving the standard protocol plus a "WASH kit", as describe in Table 1.1. Allocation was undertaken remotely by a statistician at the London School of Hygiene and Tropical Medicine. The trial evaluated a public health intervention seeking to change specific behaviours through direct engagement with participants such that blinding of participants to their allocation was not deemed possible. Randomisation was conducted remotely but the data collection team, principal investigator, and trial statistician were blinded to allocation.

*Informed Consent*

Nurses at participating health facilities assessed eligibility for participation and written consent was obtained by the senior nurse for the facility (ICP; Infirmier Chef de Poste). The designated nurse at each health facility explained the study to the caregiver using a standard Participant Information Sheet and Consent Statement that was read in Wolof or Pular according to preference. Hard copies of both documents were provided to all participant caregivers in Wolof or Pular. The consent and PIS forms are included in Appendix 4. Only eligible children for whom the parent signed and dated a consent form were enrolled in the study. In the case of illiterate participants, an independent witness signed and dated the consent form and the participant drew a cross mark in the space provided. Caregivers were informed that they could withdraw from the study subsequent to enrolment, and any data already collected and analysed were used, unless the participant requested otherwise, but no further analysis was done nor

samples kept. No incentives to participate were provided; however, any travel costs to the UREN that would not otherwise be incurred were reimbursed.

*Intervention*

Participants in both the control and intervention groups received the standard national protocol for outpatient treatment for SAM as recommended by the Ministry of Health for Senegal which covers medical treatment, nutrition treatment, and infant and young child feeding (Table 5.2). In addition to the standard protocol, participants in the intervention group received the following: a WASH kit comprising one plastic container (20L), Aquatab drinking water treatment product (quantity to treat 20L/day for one week, each week, for period of OTP, up to 8 weeks), three bars of soap, drinking water treatment promotion and demonstration at the UREN by the nurse. Follow up on the use of the product: assessment of compliance with the protocol, actual use, acceptability, safety through household visits in the course of the treatment.

Table 5.2 - Treatment received in in the control and intervention arms of the trial

| **Control** | **Intervention** |
|---|---|
| **Standard protocol for outpatient treatment of severe acute malnutrition** | **Standard protocol for outpatient treatment of severe acute malnutrition** |
| Medical treatment | Medical treatment |
| Vitamin A (at entry)<br>• 06-11 months: 100,000 IU<br>• 12-59 months: 200,000 IU<br>Albendazole/Mebendazole (at entry)<br>• 06-11 months: no/250mg<br>• 12-23 months: 200/500mg<br>• 23-59 months: 400/500mg<br>Iron/folic acid (fortnightly) | Vitamin A (at entry)<br>• 06-11 months: 100,000 IU<br>• 12-59 months: 200,000 IU<br>Albendazole/Mebendazole (at entry)<br>• 06-11 months: no/250mg<br>• 12-23 months: 200/500mg<br>• 23-59 months: 400/500mg<br>Iron/folic acid (fortnightly) |

| | |
|---|---|
| • <10kg: 60mg<br>• >10kg: 120mg | • <10kg: 60mg<br>• >10kg: 120mg |
| Nutritional Treatment | Nutritional Treatment |
| • Ready-to-use Therapeutic Food (weekly)<br>• Child Feeding sensitization (at entry) | • Ready-to-use Therapeutic Food (weekly)<br>• Child Feeding sensitization (at entry) |
| Surveillance | Surveillance |
| • MUAC measurement (weekly)<br>• Weight and height measurement (weekly) | • MUAC measurement (weekly)<br>• Weight and height measurement (weekly) |
| **No WASH Kit** | **WASH Kit** |
| | • 20L drinking water container lid and tap at admission<br>• Hygiene and drinking water treatment session at admission<br>• 3 bars of soap at admission<br>• Aquatabs to treat 20L water daily provided weekly |

### Data collection

Health registry data was recorded by attending nurses at admission and all subsequent weeks of treatment through recovery and discharge, abandonment, referral, death, and then entered into a database using ODK forms. Data entry was verified by a remote team using anonymised images of registries to extract, enter and check data for consistency. Data was collected by the research team at admission.

### Stool sample collection and analysis

At study exit (eight weeks post-admission), nurses collected a rectal swab from participants. Flocked nylon rectal swabs were eluted in 1 mL of liquid Amies solution (eSwab, Copan Diagnostics, catalogue #484CE). Approximately 40 µL of eluate was added to each of four spots on an FTA micro elute card (Qiagen, catalogue # WB120410) and air dried for a minimum of three hours before storage in individual plastic bags with desiccant. Samples were stored in the dark at ambient temperature until shipment to the London School of Hygiene & Tropical Medicine for extraction and molecular analysis.

Rectal swabs were analysed for 30 enteric pathogens using a microfluidic qPCR array card (TaqMan Array Card); the laboratory methods have been described previously (26). At LSHTM, DNA and RNA were eluted from the Whatman FTA Elute Cards following bead-beating and heat release steps. The prevalence of specific enteric infections was assessed using a custom-designed TaqMan Array Card (TAC) a microfluidic card designed to perform 48 individual quantitative PCR (qPCR) assays per sample simultaneously. The TAC has been extensively tested for quantitative detection of enteric pathogens in stool and other sample matrices (27-30). The TAC quantitatively detects 30 different enteric pathogens. Pathogens were selected based on three criteria, that they were (1) diarrhoeagenic; (2) helminths, or (3) non-diarrhoeagenic, non-helminth, but WASH-related, in that order. We selected gene targets for each pathogen of interest based on the availability of published qPCR assays previously validated on TAC and the need to differentiate between specific species, strains, subtypes or virulence factors of a given pathogen (full list Appendix 5).

### Sample size calculation and analysis

All statistical analyses were carried out in Stata, version 18 (31). For the original sample size calculation, we used the Hayes-Bennet formula for cRCTs (32). Assuming an intra-class correlation co-efficient of 0.09, and a recovery rate of 67%, based on historical data from the study area, we estimated that with 80% power, and an Alpha error of 5% a sample size of 1,720 children across 86 clusters would be sufficient for a minimum

detectable difference (MDD) in the proportion recovered rates between arms. This MDD was judged appropriate based on the findings of an earlier trial of a very similar intervention (19).

Analysis for all primary and all secondary outcomes was carried out at the individual level with adjustment for clustering within health centres. We adopted an "intention-to-treat" approach whereby data were analysed according to their allocation to either the intervention or control group, irrespective of participant response to the intervention. Child's age and gender were adjusted for a priori, and further individual-level variables adjusted for if they appeared imbalanced between the groups.

For the primary outcome of recovery, the counts and proportions of children recovering in each arm are presented, and the odds ratio for recovery in the intervention group relative to the control group with 95% confidence intervals estimated using a mixed effects logistic regression model with random effects at the health centre level to account for clustering. For weight gain, a mixed effects linear regression model was used to estimate mean differences between groups. For mortality, referral and presence of enteric pathogens, counts and proportions of children in each arm will be presented, and odds ratios with 95% confidence intervals will be estimated using mixed effects logistic regression models. For the prevalence of diarrhoea, a mixed effects logistic regression model for the prevalence of diarrhoea at four and eight weeks post-admission adjusted for differences between arms in the prevalence of diarrhoea at admission.

### Ethical approval

This study received ethical approval from the National Committee for Bio-Ethics for Senegal (Ref: SEN 19/45) and the LSHTM Research Ethics Committee (ref 17511) prior to enrolment of any study participants, and the trial was pre-registered at clinicaltrials.gov (NCT04667767). The letters granting ethical approval are included as annexes.

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# RESULTS

# 6

## Chapter 6: Enteric pathogen exposure among infants in Kisumu, Kenya

## Introduction

Despite large reductions in attributable morbidity and mortality in the last three decades, diarrhoeal diseases remain a major public health challenge in many regions of the world. In 2019, diarrhoeal diseases were estimated to be the fifth leading cause of the global burden of disease across all ages, and third overall cause among children (1). This disease burden is concentrated in low- and middle-countries (LMIC), with one in ten child deaths in these countries caused by diarrhoeal diseases (2). Diarrhoea disease aetiology episodes is often unclear but recent large studies in high-burden settings have identified a number of important pathogens responsible for moderate and severe childhood diarrhoea, spanning bacteria, viruses and protozoa (3, 4). These studies also showed significant aetiological variation between settings and age groups. Further reductions in the global burden of disease attributable to diarrhoea may be strengthened by strategies that are effective in addressing these different diseases through addressing environmental factors, such as drinking water and sanitation, as well as ensuring access to vaccines for vaccine-preventable diseases such as rotavirus, and timely clinical treatment for acute illness.

The advent and application of new molecular methods for stool-based detection of enteric pathogens provides new insights into the diversity of diarrhoegenic pathogens children are exposed to in high-burden settings. Molecular methods, using multi-target platforms for the simultaneous detection of a wider range of pathogens, are far more sensitive than traditional microbiological methods and can provide a more complete accounting of pathogen carriage in high-burden settings. Enteric pathogen detection, even where asymptomatic, is associated with important consequences. In various settings, a high burden of enteric pathogen detection has been found to be associated with adverse growth outcomes (5, 6).

Faecal-oral transmission of diarrhoeal diseases can occur through multiple environmental routes and the disease burden is generally highest in areas with poor access to safe drinking water and sanitation. Transmission by these environmental pathways can be prevented by environmental health interventions, such as improving

access to drinking water, sanitation and hygiene, and enteric pathogen carriage is generally much higher and occurs much earlier among populations living in poor environmental conditions. By contrast, in settings with near universal access to safe drinking water, sanitation and hygiene, such as Sweden, enteric pathogen carriage in early childhood is far less prevalent (7). Studies so far have mostly focused on high-burden rural settings with less attention given to informal urban settings where high population density is combined with a lack of public health infrastructure and attendant health risks.

In this study we aimed to assess the prevalence and diversity of stool-based enteric pathogen detection among infants residing in informal urban neighbourhoods of Kisumu, Kenya. To our knowledge this is the first study of its kind in this type of setting, and it provides new information to support the design of measures to prevent early childhood exposure to enteric pathogens and the resulting risk of disease and growth and developmental consequences.

## Methods

### *Study Design and Objectives*

This cross-sectional study was nested within a cluster-randomised controlled trial (cRCT) to assess the impact of a food hygiene intervention on food contamination and infant health (8). Our study has three specific objectives: first, to estimate the prevalence and diversity of diarrhoeagenic pathogens in infant stool; second, to assess the association between pathogen detection in stool and caregiver reported diarrhoea symptoms; and, third, to explore the clustering of enteric pathogens within infants. The main outcome of interest was stool-based detection of diarrhoeagenic enteric pathogens among five-month-old infants (21-23 weeks of age). Study participants were recruited across 54 clusters, each corresponding to the catchment area of a Community Health Volunteer (CHV), a health extension worker responsible for

delivering community health services to approximately 100 households. All participants were recruited to the study between March 2018 and June 2019.

*Study Setting*

The setting has been described in more detail previously (8). The study took place in two informal neighbourhoods of Kisumu, Kenya, a city of approximately 400,000 inhabitants in the Western region of Kenya. These two informal neighbourhoods – Nyalenda A and Nyalenda B – have limited access to safely managed drinking water and sanitation, and generally poor environmental conditions (9). There is a high prevalence of domestic and productive animals in these neighbourhoods with open grazing and accompanying presence of animal waste in household compounds and public spaces (10). Various studies have reported high levels of contamination in these neighbourhoods across different environmental compartments, including household drinking water (9), infant and child food (11), and soils in public spaces (12). In Kenya as a whole, and in Kisumu County specifically, diarrhoea remains a major cause of childhood disease and death (13). Drinking water, sanitation and hygiene have been identified as the leading risk factors for overall disease burden in Kisumu, accounting for over 6,000 disability-adjusted life years per 100,000 of population in 2016 (13). Recent work has implicated a broad range of bacterial, viral and protozoan pathogens as etiological factors for diarrhoeal disease morbidity and mortality in Kenya with marked variation by age group (3).

*Participant enrolment, data collection and sample collection*

Infants were enrolled at between 21 and 23 weeks of age, with a questionnaire administered and stool sample requested concurrently. A short questionnaire was administered by a study enumerator to the infant's caregiver after which the caregiver was provided with multiple diapers and a biohazard bag. The caregiver was then requested to use the diapers on the infant until the infant had defecated and stool was

captured in the diaper, then folding the diaper into the provided biohazard bag, and storing the bag a cool, dark location until the enumerator returned to the household to collect the sample the next day. Enumerators placed the bag with diapers and stool on ice packs in a cooler box and transported it to the laboratory at Great Lakes University Kisumu (GLUK). If the infant had not defecated on the day of sample collection or the stool sample was insufficient, the enumerator returned the following day, and this was continued for up to five consecutive days. Chain of Custody logs were maintained on all stool samples to laboratory receipt.

*Laboratory Analysis*

Technicians sterilized the outside of the biohazard bag in a sterilized biosafety cabinet with laminar air flow, removed the diaper from the bag, unfolded it carefully, and inspected the stool, recording evidence of water, bloody, or mucoidal diarrhoea. For analysis, a 200 mg sample of each stool sample stool from the center of the stool mass was measured on a microbalance (OHaus Corp., Parsippany, NJ) into a Zymo Shield Collection container, homogenized on a vortexer, and DNA and RNA is co-extracted using the ZymoBiomics DNA/RNA Miniprep Kit according to the manufacture's protocol (Zymo Corp., CA, USA). DNA/RNA was stored immediately in a − 20 °C freezer at GLUK until transfer to the University of Iowa for molecular analysis. A second 200 mg stool sample was transferred to a labelled sterile Eppendorf tube and stored in a − 20 °C freezer as a repository in the event that primary samples were lost, mis-labelled, or otherwise destroyed. One process negative control was prepared each day by leaving a Zymo Shield Tube open in the cabinet during stool processing, and then processing it for DNA/RNA extraction.

Stool samples were analysed for presence of the following 23 pathogens: *Aeromonas hydrophila*, *Campylobacter jejuni/C. coli, Clostridium difficile,*
Enteroaggregative *Escherichia coli* (EAEC), Enterohemorrhagic *E. coli* (EHEC) 0157, Enteropathogenic *E. coli (*EPEC), Enterotoxigenic *E. coli* LT-only toxin (ETEC-LT), Enterotoxigenic *E. coli* ST toxin (ETEC-ST), *Salmonella enteritidis, Shigella* spp., V*ibrio*

*cholerae,* Adenovirus 40–41, Adenovirus broad species, Norovirus GI, Norovirus GII, Rotavirus, *Giardia duodenalis* Assemblage A, *Giardia duodenalis* Assemblage B, *Cryptosporidium spp*., *C. hominus,* and *C. parvum*. The gene targets, forward and reverse primers for each pathogen are included in Supplementary Materials (Appendix 5). Pathogens were detected and quantified by quantitative real-time polymerase chain reaction using customized Taqman Array Cards on a ViiA7 thermocycler (Life Technologies, USA) as previously described (8) with the exception of adding 300 uM bovine serum albumin (BSA) to reduce inhibition during PCR (14, 15). A water-only qPCR negative control was analyzed every ten cards. Serial dilutions of DNA and/or RNA from quantified pathogen sources (extracts from cfu counted bacterial cultures, IDT gBlocks, and viral RNA from BEI resources) were analyzed for each batch of cards to verify assay functionality.

All raw qRT-PCR quantitative cycle (Cq) results were independently inspected and recorded by two different lab technicians. Any inconsistencies between Cq values for any given gene target were jointly reviewed to achieve agreement on the valid Cq. Any Cq <=35 were deemed positive for a pathogen gene, and otherwise were defined as negative to reduce the likelihood of false-positive misclassification. In the event that pathogen genes are detected in process negative controls, monoplex PCR was used to verify that detection represented true contamination and, if confirmed, stool samples processed on that day were considered non-determined (ND) for the related pathogen. Detection of most pathogenic *E. coli* involved screening for two different genes, which could be present individually or jointly in a bacterium. For reporting aetiological detection patterns, we adhered to the common practice of defining pathotypes based upon gene combinations that distinguish between host specificity or risk for severe diarrhoea. Specifically, any stool with *bfpA,* with or without *eae*, was defined as typical EPEC but *eae* alone was atypical EPEC. Any stool with *est,* with or without *elt*, was defined as ETEC ST but *elt* alone was ETEC LT. Any stool with *aacA* and/or *aaiC* was defined as EAEC.

*Sample size and statistical analysis*

The target sample size was determined by the Safe Start trial and represents the total enrolled participants for that study with that sample justification for the trial previously described (8). Only those infants with complete outcome data were included in analysis. Detected pathogens were summarised in three ways: a binary indicator of whether at least on pathogen was detected; a count of the number of pathogens detected; and lastly a binary indicator for each individual pathogen. Diarrhoea was assessed using a binary indicator of whether or not the caregiver reported that the infant had had diarrhoea in the week preceding stool collection.

Descriptive tables and figures were produced to present summary information on participant characteristics, and frequency of pathogen detection and symptoms. To assess the association of overall burden of infection with symptoms two regression were fitted, both with a binary indicator of self-reported diarrhoea in the previous week as outcome. The first model included a binary indicator of presence of at least one detected pathogen per child as the single predictor; the second included the overall count of detected pathogens per child. To assess the association of each individual pathogen with symptoms, univariable logistic regressions were fitted with each pathogen in turn as the single predictor variable. All regressions were generalised estimating equations (GEE) which accounted for clustering at the level of study clusters which corresponded to the catchment areas of Community Health Volunteers from within which infants were recruited.

Clustering of infections within infants was assessed in two stages. First, a factor analysis was considered to assess whether there were underlying factors explaining shared variance between the factors. The first step was to inspect all pairwise Pearson correlations to assess whether there existed substantial levels of correlation (this was assessed to be over $r = 0.30$). This was used to inform whether it would be possible to carry out a factor analysis. Second, a cluster analysis was carried out using the binary indicators for each pathogen. This was repeated using ‘cq’ concentrations. For analysis using binary variables the ‘k-medoids’ clustering algorithm was used, with the Gower distance. For the analysis using the continuous measures of concentration the ‘k-

means' algorithm was used with the Euclidean distance. The within cluster sum of squares was used to inform the optimal number of clusters. The average silhouette was used to assess the fit of the clusters, as well as inspection of cluster characteristics. All statistical analyses were carried out in R using packages 'geepack' (16) and 'factoextra' (17).

### *Ethics Statement*

The research team obtained written informed consent from the infant's caregiver before enrolment in the study. The data presented here were collected as part of the Safe Start trial a cluster-randomized controlled trial of an infant food hygiene behavior change intervention (8). The trial was pre-registered on the clinicaltrials.gov registry (ID: NCT03468114) and ethical approval for the study was provided by the research ethics committees of Great Lakes University, Kisumu (Ref. No. GREC/010/248/2016), the London School of Hygiene and Tropical Medicine (Ref. No. 14695), and University of Iowa (IRB ID 201804204). The documents confirming ethical approval for the trial are included as appendices (Appendix 3).

## Results

### *Participant Characteristics*

The caregivers of 888 infants aged between 21 and 23 weeks of age gave consent for their enrolment in the Safe Start trial. Of these, 109 (12.3%) were subsequently excluded from this study as either stool sample or caregiver reported diarrhoea were not obtained.

779 infants were included of whom 397 (51%) were female and 382 (49%) male, and most infants were reported to have received vaccination for Rotavirus (92.8%), and to be currently breastfed (99.5%) (Table 6.1). Infant caregivers were mostly female (98.5%),

and mostly the mother of the infant (96.7%), with almost all caregivers having completed at least primary education but only a minority having completed higher level education (15.9%) (Table 6.1). The majority of caregivers reported handwashing with soap in the last day (98.8%), and access to improved sanitation and drinking water was 86.4% and 98.8% respectively for infant households. 122 caregivers (15.7%) reported regular contact with animals, and 99 (83.5%) of those reported that animals slept inside the house (Table 6.1).

*Table 6.1 - Study participant characteristics*

| | **N (%)** |
|---|---|
| | |
| **Total participants** | 779 (100.0) |
| **Infant sex** | |
| Female | 397 (51) |
| Male | 382 (49.0) |
| **Currently breastfed** | |
| Yes | 775 (99.5) |
| No | 3 (0.4) |
| Missing | 1 (0.1) |
| **Eaten food in last 24 hours** | |
| Yes | 38 (4.9) |
| No | 740 (95.0) |
| Don’t know | 1 (0.1) |
| **Rotavirus vaccination** | |
| Received | 723 (92.8) |
| Not received | 8 (1.0) |
| Vaccination card missing | 48 (6.2) |
| **Caregiver sex** | |
| Female | 767 (98.5) |
| Male | 12 (1.5) |
| **Caregiver relation to infant** | |
| Mother | 753 (96.7) |
| Father | 12 (1.5) |
| Other | 14 (1.8) |
| **Caregiver education** | |
| Higher | 124 (15.9) |
| Secondary | 350 (44.9) |

| | |
|---|---|
| Primary | 304 (39.0) |
| None | 1 (0.1) |
| **Caregiver years of residence in current household** | |
| ≤1 year | 264 (33.9) |
| 2-3 years | 145 (18.6) |
| 3-4 years | 93 (11.9) |
| >4 years | 277 (35.6) |
| **Caregiver reported washing hands with soap in last day** | |
| Yes | 770 (98.8) |
| No | 9 (1.2) |
| **Household has access to improved sanitation facility** | |
| Yes | 673 (86.4) |
| No | 106 (13.6) |
| **Household has access to improved drinking water source** | |
| Yes | 770 (98.8) |
| No | 9 (1.2) |
| **Caregiver reported contact with animals in previous day** | |
| Yes | 122 (15.7) |
| No | 657 (84.3) |
| **Animals sleep inside house of infant** | |
| Yes | 91 (11.6) |
| No | 688 (88.4) |

*Prevalence and Diversity of Enteric Pathogen Detection Among Infants*

At least one of the 23 target pathogens was detected in the stool of 696 (89.3%) of the 779 infants included in the study, with multiple pathogens (>1) detected for 523 (67%) of infants, and a median number of pathogens per child of 2.00 (IQR 1.00, 3.00) (Table 2; Figure 6.1). The most frequently detected pathogen was EAEC with 560 (71.9%) infants, and the next three most frequently detected pathogens were all pathogenic E. coli with tEPEC, aEPEC and ETEC-LT present in 163 (20.9%), 154 (19.8%) and 115 (14.8%) of infants respectively. The most frequently detected viruses were Adenovirus, and Norovirus GII and GI, detected in 39 (5.0%), 128 (16.4%) and 87 (11.2%) infants respectively. Detection of protozoal pathogens was less frequent, with Cryptosporidium detected in 55 (7.1%) and Giardia, both types A and B, detected in no infants.

*Table 6.2 - Pathogen detection in infant stool*

| | **n (%)** |
|---|---|
| **At least one pathogen detection (%)** | |
| Yes | 696 (89.3) |
| No | 83 (10.7) |
| **Diarrhoea in previous week reported by caregiver (%)** | |
| Yes | 118 (15.1) |
| No | 661 (84.9) |
| **Water or blood observed in stool (%)** | |
| Yes | 329 (42.2) |
| No | 450 (57.8) |
| **Number of pathogens detected per infant (%)** | |
| 1 | 173 (24.9) |
| 2 | 195 (28.0) |
| 3 | 168 (24.1) |
| 4 | 108 (15.5) |
| 5 or more | 52 (7.5) |
| **Median number of pathogens (Number [IQR])** | 2.00 [1.00, 3.00] |
| **Frequency of Pathogen Detection in infants (%)** | |
| Aeromonas | 23 (3.0) |
| *Campylobacter jejuni/C. coli* | 70 (9.0) |
| *Clostridium difficile* | 59 (7.6) |
| enteroaggregative *E. coli* (EAEC) | 560 (71.9) |
| enterohemorrhagic *E. coli* (EHEC) 0157 | 26 (3.3) |
| typical Enteropathogenic *E. coli (*tEPEC) | 163 (20.9) |
| atypical Enteropathogenic *E. coli* (aEPEC) | 154 (19.8) |
| enterotoxigenic *E. coli* LT toxin (ETEC-LT) | 115 (14.8) |
| enterotoxigenic *E. coli* ST toxin (ETEC-ST) | 71 (9.1) |
| *Salmonella enteritidis* | 21 (2.7) |
| *Shigella* spp. | 31 (4.0) |
| V*ibrio cholerae* | 1 (0.1) |
| Adenovirus 40–41 | 39 (5.0) |
| Adenovirus broad species | 130 (16.7) |

| | |
|---|---|
| Norovirus GI | 87 (11.2) |
| Norovirus GII | 128 (16.4) |
| Rotavirus | 38 (4.9) |
| *Giardia duodenalis* Assemblage A | 779 (100.0) |
| *Giardia duodenalis* Assemblage B | 3 (0.4) |
| *Cryptosporidium spp.* | 55 (7.1) |
| - *C. hominus* | 8 (1.0) |
| - *C. parvum* | 1 (0.1) |
| - *Cryptosporidium 18s* | 47 (6.0) |

*Association between Pathogen Detection and Symptoms*

The association between pathogen detection and diarrhoeal disease symptoms was assessed using a one-week period prevalence of diarrhoea reported by the caregiver at time of stool collection and using the World Health Organisation definition of, “the passage of three or more loose or liquid stools per day”. One hundred and eighteen children (15%) were reported by their caregiver to have had diarrhoea in the week preceding sample collection (Table 6.2) of which 109 (92%) had at least one pathogen detection. Of the 696 (89.5%) infants with at least one pathogen detected 16% were symptomatic (Table 6.2).

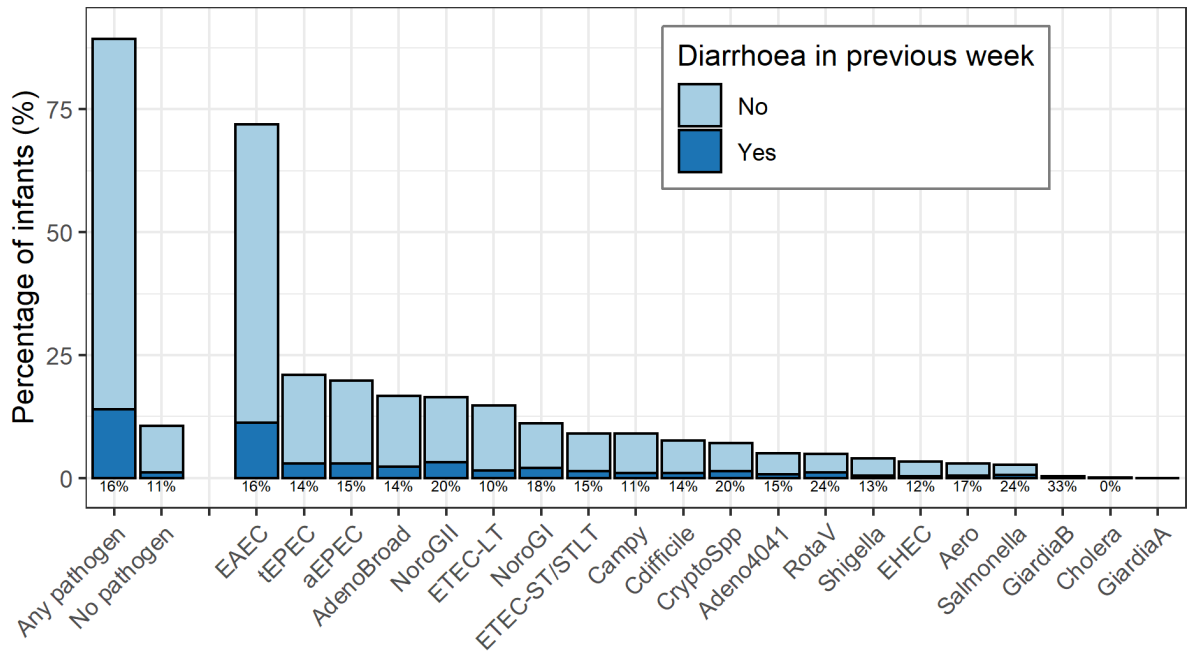


*Figure 6.1 - Frequency of symptomatic and asymptomatic pathogen detection in infants*

The results of the GEE logistic regression of symptoms on presence of at least one pathogen is shown in Table 3. There was no evidence that the detection of one or more pathogens was associated with odds of caregiver-reported diarrhoea symptoms (odds ratio: 1.53, 95% CI 0.74, 3.14). The model with integer count of number of pathogens as a predictor is shown in Table 6.3. There is no evidence that a greater number of detected pathogens was associated with increased odds of caregiver reported diarrhoea (OR 1.03, CI: 0.90, 1.18). The results of multiple univariable regressions with each individual pathogen as predictor are shown in Table 6.3 and there is no evidence that detection of any individual pathogen was associated with increased odds of self-reported symptoms.

*Table 6.3 - Association between detection of one or more pathogens, number of pathogen detected, and detection of individual pathogens and diarrhoeal symptoms*

| **Outcome** | **Odds Ratio** (95% confidence interval) | **P-value** |
|---|---|---|
| Presence of at least one enteric pathogen | 1.53 (0.74, 3.14) | 0.25 |
| Number of pathogens detected | 1.03 (0.90, 1.18) | 0.68 |
| ETEC-LT | 0.61 (0.33, 1.16) | 0.13 |
| Campylobacter | 0.7 (0.33, 1.51) | 0.37 |
| Shigella | 0.82 (0.28, 2.4) | 0.72 |
| Cdifficile | 0.87 (0.4, 1.88) | 0.72 |
| AdenoBroad | 0.88 (0.52, 1.51) | 0.65 |
| tEPEC | 0.9 (0.55, 1.48) | 0.68 |
| aEPEC | 0.98 (0.6, 1.61) | 0.93 |
| Adeno4041 | 1.02 (0.42, 2.49) | 0.97 |
| ETEC-ST | 1.03 (0.52, 2.03) | 0.93 |
| EAEC | 1.17 (0.75, 1.84) | 0.48 |
| NoroGI | 1.3 (0.73, 2.34) | 0.37 |

*Clustering of detected pathogens within infants*

To explore whether certain pathogens were clustered within infants, we employed two strategies, a factor analysis to identify underlying factors explaining co-variance, and then a cluster analysis to identify sub-groups with similar patterns of pathogen detections. For the factor analysis, we removed two pathogens were removed for having very high correlations with another, similar, pathogen (Crypto18S, ETECLTonly) and one for having no instances in the data (GiardiaA). The correlation matrix was inspected. Most correlations were too low for a factor analysis to be viable: only three pathogens had a correlation greater than 0.3. Therefore, a factor analysis was not carried out and it was concluded that no shared causes of variation between groups of pathogens was found. The total within cluster sums of squares decreased with the number of clusters. After five clusters, there was a relatively small decline in WSS. The five-cluster solution was inspected first. The average silhouette was small (0.18), indicating a poor fit. Other cluster solutions (3,4,6) had similarly low values. This was also found in the clusters derived using binary data. Summary statistics for each cluster were found to describe the pattern on infections. It was observed that the clusters were dominated by the most common pathogens and tended to replicate the most common pairs of pathogens. There was no pattern of clustering in the low frequency pathogens. No satisfactory cluster solution was found. As no distinct groups of infants with similar patterns of detected enteric infections were identified, we found no evidence for clustering of particular pathogens within infants.

## Discussion

This study assessed the community prevalence of enteric pathogen detection of young infants in a high-burden informal urban setting in sub-Saharan Africa, using quantitative multiplex PCR. Previous studies have generally - but not exclusively - focused on older children and most studies have been conducted in rural settings with markedly different environmental characteristics. There are three main findings. First, that among infants

of only five months of age, there is already a high prevalence of pathogen detection and co-detection. Second, a diverse range of diarrhoeagenic enteric pathogens were detected in this young population; and third, pathogen detection was not associated with caregiver reported diarrhoea and the majority of detections were asymptomatic. Although we investigated pathogen clustering, we found no evidence for clustering of certain pathogens with infants.

Our study of infants residing in informal urban neighbourhoods of Kisumu, Kenya, found that 90% of infants were positive for at least one of the 23 targeted enteric pathogens detected, and 67% were positive for two or more pathogens. The 23 pathogens were selected on the basis that they are well-established diarrhoeagenic agents among populations with a high burden of moderate and severe diarrhoea (18, 19). The prevalence of enteric pathogen detection was high Although previous studies have generally focused on the wider age interval of children under five years of age, some provide sub-group results for younger age intervals closer to the age of infants in our study. One study in Maputo, Mozambique reported that, among infants aged 1-11 months of age, 71% were positive for one or more of the assessed pathogens (n=15) using a qualitative multiplex PCR assay. Within that age interval, 33% of infants were positive for two or more pathogens. Both the prevalence of one or more detections, and the prevalence of co-detections, was much lower than that found in our study. That study however assessed the presence of fewer pathogens and did not include, for example, EAEC and EPEC which were the most frequently detected in our study which may explain why we found a higher overall detection prevalence. Another study in Dhaka, Bangladesh, reported the mean number of pathogens detected in the first year of life (1-11 months) as 3.3 (IQR: 2-4)(20). The longitudinal design of that study with weekly sampling prevents a direct comparison with our own finding of a mean number of pathogens detected at a single time point (5 months of age) of 2.0 (IQR: 1-3) but both are strikingly high.

Studies estimating the community prevalence of enteric pathogen detection in low burden settings in high-income country settings are rare. One study conducted among children in daycare facilities in Upsala, Sweden, which reported a detection prevalence

of less than 1% so dramatically lower than our results or the other studies in high-burden settings discussed above. The study in Dhaka discussed above included a comparison cohort in Virginia, US, and reported far lower detection rates among children there compared to Dhaka (median of 0.5 detections in first year of life, IQR: 0-1)(20). Other studies using similar detection methods – stool-based multiplex PCR - in high settings were identified but these were predominantly in clinical settings and or concerned specific population groups, such as men with AIDS, in the US (21). One community-based study of all ages in Australia, reported that pathogen detection was rare in asymptomatic individuals who had not sought medical care (22). More estimates for the community prevalence of enteric pathogen detection among children and infants in high-income settings with their environmental settings – including access to WASH services - would provide a useful point of comparison. One study in Vellore, India, though, compared enteric pathogen detection rates directly between a low-income and a high-income cohort in the same area (23). One cohort resided in a neighbourhood described as, "semi-urban, partly formal (formerly described as slums)" with poor water and sanitation services, and the second in the residential campus of the Christian Medical College of Vellore with good housing, a continuous supply of piped treated drinking water and good sanitation. At 3-6 months of age, they found a marked difference in the prevalence of bacterial infections with 55.6% of children in the "semi-urban" cohort positive for at least one of the target bacteria compared to 14.3% among the medical campus cohort.

Previous studies using similar methods in populations with a high burden of diarrhoeal disease have identified a wide range of pathogens that are similar to those we identified(19, 24). The four most frequently detected pathogens in this cohort of infants were all bacterial pathogens: EAEC (71.9%), tEPEC (20.9%), aEPEC (19.8%) and ETEC-LT (18.5%) (Table 2). These pathogenic *E. coli* are well-established causes of childhood diarrhoea. EAEC was the most frequently detected pathogen among infants in this study and has been reported as similarly prevalent in other high burden settings (20, 25-27). Whilst almost 90% of infants had at least pathogen detected, only 15% of infants had experienced diarrhoea within the previous week, such that the majority of pathogen detections were asymptomatic. The presence of no individual pathogen was found to be

associated with symptoms and neither was the detection of any pathogen (one or more pathogens detected) or the number of pathogens. The aim of this study was not to assess the aetiology of diarrhoea, but the high proportion of detections among asymptomatic infants is notable. Studies designed to assess the aetiology of diarrhoea using case-control (24) or cohort designs (19) have at the same time highlighted the high rates of asymptomatic carriage.

The study has several limitations. Firstly, diarrhoea was reported by caregivers and therefore at risk of reporting bias. A counterweight to this was that information was collected on the physical state of stool samples at collection that provided more objection information on whether stools were watery, but this does not of course permit assessment of the frequency that stools were passed by the child in the previous 24 hours as per the WHO definition of diarrhoea. A second limitation is that whilst we asked caregivers whether the infant was currently being breastfed, it was not possible to ascertain whether breastfeeding was exclusive or mixed. We asked separately whether food had been given to the infant in the previous 24 hours for which only a small proportion responded they had (4.9%). As exclusive breastfeeding is actively promoted by community health workers through to six months of age it is possible that caregivers may be disinclined to report other feeding. Lastly, in a high burden setting such as this some caution is required in the interpretation of these detection results. Firstly, the high burden of enteric pathogen exposure combined with high sensitivity of the multiplex PCR methods used increases the risk of detected residual DNA/RNA from previous infections (28). In addition, and with regard to the relative detection rates for different pathogens, it is important to note that the period of shedding can vary significantly by pathogen which will in turn affect the probability of detection at a single timepoint (29). It is important therefore to interpret these results only as the detection of pathogen in stool which confirms prior exposure to these pathogens only and does not provide a basis for diagnosis where detection is in symptomatic individuals nor a basis for estimating the aetiological contribution of different pathogens within this population. For the purposes of environmental health though understanding exposure is paramount as interventions are designed to modify the environment so as to prevent exposure rather than treating infection and disease.

## Conclusions

Our results demonstrate that, from a young age, infants living in high density informal areas with limited public health infrastructure are exposed to a diverse range of enteric pathogens such that even at six months of age most are positive for at least one enteric pathogen. The clinical consequences of this are unclear as there was no association between pathogen detection and diarrhoeal disease symptoms. However, the high burden of enteric pathogen detection at only five months of age confirms the need for measures that can prevent exposure within this vulnerable group.

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# 7

## Chapter 7: Effectiveness of a food hygiene intervention on enteric pathogen exposure among infants in Kisumu, Kenya

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# RESEARCH PAPER COVER SHEET

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|---|---|---|---|
| **Student ID Number** | 279050 | **Title** | Mr |
| **First Name(s)** | Oliver | | |
| **Surname/Family Name** | Cumming | | |
| **Thesis Title** | The influence of water, sanitation and hygiene conditions on enteric pathogen exposure and related health outcomes in vulnerable children – evidence from two trials | | |
| **Primary Supervisor** | Prof. Tanya Marchant | | |

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| | |
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| Stage of publication | **Not yet submitted** |

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| | |
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| For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary) | I conceived this study with Dr Jane Mumma. I secured funding for this work and designed the study. With support from the trial statistican, I analysed the data, prepared the tables and figures and wrote the original draft of the manuscript. |

**SECTION E**

| | |
|---|---|
| **Student Signature** | [redacted] |
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| | |
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| **Supervisor Signature** | [redacted] |
| **Date** | 03.04.2025 |

Abstract

**Background**

Exposure to microbially contaminated food in early childhood can lead to disease and growth and developmental consequences. The aim of the "Safe Start" trial was to assess the effect of a food hygiene intervention on stool-based enteric pathogen detection and diarrheal disease among infants in a low-income informal urban neighbourhood of Kisumu, Kenya.

**Methods**

We conducted a cluster-randomized trial with 54 clusters, representing the catchment areas of Community Health Volunteers (CHVs), randomly assigned at a ratio of 1:1 to an intervention or active control, and a total of 823 infants were recruited on a rolling basis at 22 weeks of age and then followed up through 37 weeks of age. The study investigators, and laboratory personnel were blinded to the treatment assignment. The intervention was co-designed with, and delivered through, local public health structures and targeted four key food hygiene practices: (1) hand washing with soap before infant food preparation and feeding; (2) bringing all infant food to the boil before feeding, including when reheating or reserving; (3) storing all infant food in specific sealed containers; and, (4) using only designated utensils for infant feeding. The active control arm received the same number of visits by CHVs who delivered standard public health messaging. The primary outcome was determined by the detection of one or more enteric pathogens in the infant's stool at 37 weeks of age (± 1 week). The secondary outcome was measured by assessing the number of caregiver-recorded days a child had diarrhoea through follow-up (between 22 and 37 weeks of age (± 1 week).

**Findings**

At least one out of the 23 pathogen associated genes assessed was detected in 96.5% and 97.3% of the stool of participants in the intervention and control arms respectively. There was no evidence that the intervention reduced the odds of having at least one enteric pathogen detected in the stool. Although there was significant missing data, we found a lower longitudinal prevalence of self-reported diarrhea in the intervention arm of

the Safe Start trial. This was robust to a sensitivity analysis used to account for missing data in the self-reported measures.

**Interpretation**

This intervention to improve food hygiene practices by infant caregivers delivered by trained agents working with CHVs had no effect on the detection prevalence of enteric pathogens among infants. The prevalence of diarrhoea was 70% lower in the intervention arm after 37 weeks follow-up although imputation was required to account for significant missing data. The failure to reduce enteric pathogen detection suggests that larger scale, more comprehensive interventions are likely required to mitigate the myriad environmental hazards in complex urban settings such as these.

**Funding**

This study was funded by the Foreign Commonwealth and Development Officer of the United Kingdom.

## Introduction

Diarrhoeal diseases are generally caused by exposure to bacterial, viral and parasitic enteric pathogens, and remain a major global health concern, responsible for over a million deaths each year (1) . Beyond symptomatic disease, asymptomatic infection by - or carriage of – these same enteric pathogens is associated with adverse growth and development outcomes even in the absence of diarrhoea (2). The risk of exposure to enteric pathogens is likely high in informal urban settlements due to poor public health infrastructure, and especially unsafe sanitation and drinking water, high population density, and pervasive poverty.

A major risk factor for diarrhoeal disease – and exposure to enteric pathogens – is contaminated food. Diarrhoeal disease accounts for over half of the approximately 420,000 foodborne deaths that occur each year (3), and most of the leading global foodborne hazards identified by the World Health Organisation are diarrhoeagenic pathogens, including non-typhoidal *Salmonella enteric*a, Enteropathogenic *Escherichia coli* (EPEC), Norovirus and *Campylobacter* (4). Children bear approximately 40% of the foodborne global burden of disease, with much of this attributable to diarrhoeal disease (5). Children are particularly vulnerable in the "complementary feeding" period when children transition from exclusive breastfeeding to solid foods. At this point, their exposure to pathogens via food - as well as other environmental media - increases whilst conferred maternal immune protection wanes and their own immune system is not fully developed.

Various studies conducted in settings with a high burden of diarrhoeal disease have shown that infant food is often highly contaminated (6-11). Identified risk factors for contamination of infant food include unsafe water and sanitation services, lack of access to hygiene facilities, and the presence of animals, among other broader social determinants. Using the Hazard and Critical Control Point (HACCP) methodology (12), studies have identified critical control points (CCPs) – steps during the food preparation process where controls can be applied to reduce food safety hazards – to prevent microbial contamination of infant or child food. Several CCPs have been consistently

identified, including: safe handling of food, including hand hygiene and serving (7, 10, 11, 13, 14), cooking and re-heating to boiling (7, 10, 14-16) , and the safe storage of food (7, 10, 14, 15).

There has been limited research, however, on the design and evaluation of interventions to reduce foodborne exposure to enteric pathogens in young children by targeting related hygiene practices during infancy. There have been only four trials of dedicated infant – or complementary feeding - food hygiene interventions reporting effects on contamination of food (6-9) and only two reporting effects on diarrhoea (8, 17). With one exception in a low-density peri-urban area (6), these have all been in rural settings. There have been no trials to date evaluating the effect of an infant food hygiene intervention on stool-based enteric detection. The aim of this study was to assess the effect of a food hygiene intervention targeting infants' environment and food hygiene-related behaviours, delivered at the household in an urban informal settlement, on enteric pathogen detection in stool and diarrhoea among infants.

## Methods

*Study design and setting*

This cluster-randomized controlled trial (cRCT) was conducted in the two contiguous wards of Nyalenda A and B, in the informal peri-urban neighbourhoods of Kisumu, the third largest city in Kenya. A cluster was defined as the catchment area of a participating Community Health Volunteer (CHV). Ninety-four CHVs across the Nyalenda A and B wards were screened for participation in the trial and 54 were selected to participate. Selection was made in consultation with the Ministry of Health (MoH) of Kisumu County based on an assessment of whether the records for eligible CHVs were up to date and whether the CHV was active with a sufficient number of identified households within their catchment. We adopted a cluster-based design to build on the existing structure of the health system through which such an intervention targeting infants would be delivered but it also reduced the risk of contamination between intervention and the control arms as the catchments were clearly defined. We hypothesized that a food hygiene intervention targeting infants as they transitioned

from exclusive breastfeeding to consumption of solid foods would prevent early foodborne exposure to enteric pathogens and thereby reduce enteric infection and diarrhoea at a critical stage in childhood.

The trial received ethical approval from the Great Lakes University of Kisumu (GLUK) Research Ethics Committee (Ref: GREC/010/248/2016), London School of Hygiene and Tropical Medicine (LSHTM) (Ref: 14695), and University of Iowa (Ref: 00000099). The trial was pre-registered at the clinicaltrials.gov registry (NCT03468114) and the protocol has been published (11).

*Participants*

Infants were enrolled from the selected CHV catchment areas by the research team with support from the CHVs. Eligible infants were first identified by CHVs and their primary caregiver was invited to participate in the study. If the caregiver expressed interest in participating, a member of the research team verified that the infant met the eligibility criteria and obtained written informed consent from the caregiver. Participants were only enrolled if caregivers confirmed they intended to remain resident in their current housing for the subsequent five months. Study participants were infants enrolled at 22 weeks (± 1 week) of age, with rolling enrolment between March 2018 and January 2019. A seven-digit identifier was generated for the household of each enrolled infant, and barcodes were created to link participants to their environmental and clinical samples using the TBarCode software (Microsoft).

*Randomization and masking*

Allocation of the 54 eligible CHVs was done remotely by an independent statistician at the Clinical Trials Unit of the London School of Hygiene & Tropical Medicine (LSHTM). The 54 active CHVs were randomly allocated 1:1 to the control or intervention arms of the trial, and the list communicated directly to the study coordinator responsible for supervising the enumerators and interacting with the CHVs. Given the nature of the intervention – a public health intervention at the household level involving provision of products and health promotion activities - it was not possible to blind the participants, the research team collecting data nor the CHVs to the allocation. The study

investigators, the laboratory staff, the trial statistician were all blinded to treatment assignments.

*Procedures*

Design of the intervention

Following initial consultation with the Ministry of Health for Kisumu, we undertook a series of formative studies in a similar adjacent neighbourhood within Kisumu City which showed low rates of handwashing and poor food hygiene practices around preparation, feeding, and re-heating of infant food among caretakers(18-20). Furthermore, we conducted a formative microbiological assessment of infant food quality and confirmed that a majority (62%) of infant food samples were positive for at least one of the enteric pathogens assessed (20, 21). A pilot intervention was then designed using the Behavior-Centered Design approach and tested with infant caregivers in adjacent communities using the Trials of Improved Practice methodology which allows for iterative development based on user feedback (22).

Intervention

The “Safe Start” intervention has been described previously (23); in brief, it combined environmental modification and motivational messaging targeting four food hygiene-related behaviors of handwashing before food preparation and feeding, bringing the infant food to the boil before feeding, safe storage of all infant food, and use of designated feeding utensils. The environmental modification component was designed to facilitate changes in food hygiene practices and comprised a 20-liter bucket with lid and a tap for storing water for handwashing, 500ml liquid soap for handwashing, two deep and two rectangular storage containers with lids for storing infant food, and infant feeding utensils (a small bowl, a baby cup and a small plastic lined spoon). The motivational messaging targeted the motive of nurture with images imprinted on customized wall-calendars and place mats. The first message was titled ‘Happy Baby’ and depicted the relationship between safe food and a happy baby, and the second message was ‘Successful Child’. As described previously (23), the intervention was delivered in four

visits: visit one (at 23 weeks of age, ± 1 week ) sensitised the caregiver on the importance of food hygiene during the weaning period; visit two (25 weeks of age, ± 1 week) delivered and promoted the food hygiene materials, and 'Happy Baby' messaging; visit three (29 weeks of age, ± 1 week) reinforced key messages and allowed feedback and discussion with the caregiver; and, visit four (32 weeks of age, ± 1 week) which introduced the "Successful Child" messaging.

Delivery in the intervention and control arms

In the intervention arm, CHVs delivered the standard outreach for children of this age – e.g. exclusive breastfeeding up to six months of age and the appropriate types of food for weaning - but with addition of the intervention described above. To reduce the additional time burden on CHVs and to standardize the delivery of the intervention, CHVs were accompanied by trained agents using detailed delivery scripts with talking points for each visit. This has been described in detail previously (23) but visits were interactive with discussions, demonstrations and question and answer sessions from both sides to ensure that caregivers had understood the delivery process and how to use each item correctly.

The trial incorporated an active control arm ensuring that control households were visited at the same frequency as intervention households and thereby mitigate potential bias associated with more frequent contact with health workers. Trained agents assigned to households in the active control arm carried out the standard CHV-type activities but focused only on the standard public health activities of the Ministry of Health. Households in the active control arm also received wall calendars but without the food hygiene and infant health messages and the caregivers in the active control arm were also instructed to record days with diarrhoea.

*Outcomes*

The primary outcome for the study was the prevalence of enteric infection at age 37 weeks (±1 week). We define the prevalence of enteric infection as the presence of one or more enteric pathogens in child stools based on the detection of 23 genetic markers for specific enteric bacteria, viruses and protozoan all of which have been previously described (23). The secondary outcome was the prevalence of diarrhoea; defined as the number of days a child has diarrhoea between 22 and 37 weeks of age (± 1 week). The tertiary outcome was child mortality, defined as any infant death occurring between 22 and 37 weeks of age (±1 week). In addition, the study assessed the effectiveness of the intervention by measuring changes in specific food practices and in bacterial contamination of infant food.

*Data and sample collection*

Questionnaires were administered to caregivers by enumerators during the baseline (22 weeks of age, ± 1 week), midline (32 weeks of age, ± 1 week) and endline (37 weeks of age, ± 1 week) visits. Data were collected using a tablet-based ODK form (Ref) and data uploaded to a central server each day and external checks performed. The baseline survey included questions on general household information, WASH access, infant health, and animal contact, while the midline and endline questionnaire included questions on household information and infant health.

Food and stool samples were collected at the midline and endline respectively. The research team collected a sample of food cooked for the infant by the caregiver. The caregiver was asked to place a sample of food in a sterile WhirlPak bag by the same means as she would feed a child (e.g. spoon, hands). Samples were labelled (date, time and study identification number), placed immediately into a cooler box, maintained at < 10 °C with ice packs, and then transported to the laboratory for analysis. Caregivers were provided with several diapers during the baseline and endline visits to facilitate stool sample collection. Once the infant had defecated, the caregiver was instructed to place the soiled diaper in a biohazard bag and to keep this in a cool place until the enumerator returned to collect it the following day. In cases where the infant did not defecate within 24 hours, or the diaper was not bagged, enumerators returned on

subsequent days to a maximum of five days. Infants identified as possibly unwell either by the fieldworker or CHV during study or routine visits by the CHV, were referred to appropriate health facilities for treatment.

*Laboratory analysis*

Food samples were analysed as previously described (23) by enumerating *Enterococcus*, a bacterial indicator of faecal contamination. In brief, 1 ml (mL), 0.1 mL, and 0.01 mL dilutions of liquid foods were filtered through 0.45 $\mu$ m pore-size membrane filter (Millipore Corp., Bedford, MA, USA), and the filters cultured overnight on Slanetz & Barley Enterococcus Medium (OXOID CM0377). For solid foods, five grams were homogenized with 45 mL of sterile phosphate buffer saline (PBS), and 10 mL, 1 mL, and 0.1 mL dilutions filtered and cultured on *Enterococcus* agar plates. Plates were incubated at 41 °C ± 0.5° for 24 h. and then all light and dark red colonies counted as Enterococcus and expressed as colony forming units (CFU) present per gram of food sample. A 10 ml volume of PBS used to re-suspend solid food samples and wash membrane filters was processed each day as a negative control.

A 200 mg sample of each stool sample was measured into a Zymo Shield Collection container and DNA and RNA co-extracted using the ZymoBiomics DNA/RNA Mini kit according to the manufacture's protocol (Zymo Corp., CA, USA). DNA/RNA was immediately stored in a -20°C freezer until transfer to the University of Iowa for molecular analysis. A second 200 mg stool sample was transferred to a labelled sterile Eppendorf tube and stored in a -20°C freezer as a repository in the event that primary samples were lost, mislabelled, or otherwise destroyed. All stools were processed in sterilized biosafety cabinets with laminar air flow, and one process negative control prepared each day by leaving a Zymo Shield Tube open in the cabinet during stool processing, and then processed for DNA/RNA extraction. Pathogen targets were detected and quantified by quantitative real-time polymerase chain reaction using customized Taqman Array Cards on a ViiA7 thermocycler (Life Technologies, USA) as previously described with the exception of adding 300 uM bovine serum albumin (BSA) to reduce inhibition during PCR. Outcomes were defined as the pathogen-specific presence and concentration of individual pathogens, as well as the presence and

diversity (sum of pathogen types) of all pathogens. Concentrations of individual pathogens per gram of stool are estimated by comparison of cycle thresholds of pathogen specific genes against standard curves for each reference of interest. In the event, that pathogen genes were detected in process negative controls, monoplex PCR was used to verify that detection was true contamination. If negative controls were contaminated, the stool samples processed on the same day as the negative control were considered non-determined (ND) for the related pathogen.

*Statistical analysis*

We used a standard approach (24) for calculating sample size for cluster-randomised controlled trials, as described previously (23), and based our assumptions on published estimates for the prevalence of enteric pathogen detection (25) and diarrhoeal disease (26), and the effect size for similar early childhood interventions. With a total sample size of 750 children (375 intervention, 375 controls) across 50 clusters (25control/25 intervention) and with an anticipated intra-class correlation co-efficient (ICC) of 0.01, we estimated that we would have 80% power at a 5% level of significance to detect a minimum difference of 10% in the enteric pathogen detection prevalence between arms. We enrolled 54 clusters – versus the 50 required in our calculation - to allow for potential drop-out of CHVs from the trial.

All analysis was carried out on groups as randomised ('intention-to-treat') and accounted for the nature of the distribution of the relevant outcome and the results are presented as appropriate effects sizes (difference in means between arms; risk ratios) with a measure of precision (95% confidence intervals). Generalised estimating equations (GEE) were used to account for clustering and analyses adjusted for difference at baseline by the inclusion of the cluster mean of the outcome as a covariate in statistical models. Unadjusted and adjusted results are presented for all analyses, with covariates in adjusted analyses specified *a priori*.

The primary outcome was enteric pathogen detection defined as the detection of one or more enteric pathogens in infant stool at 37 weeks of age (± 1 week). For this binary outcome a logistic regression was fitted with generalised estimating equations GEE) to

account for the possible correlation within CHV, with an exchangeable correlation structure and robust standard errors. In adjusted models, the cluster mean prevalence at baseline was included as a covariate.

The secondary outcome was the longitudinal prevalence of carer-reported diarrhoea as recorded on the two calendars provided to caregivers. The difference between the intervention and control arm in the rate of self-reported diarrhoea (days per month) was the estimate of interest. Since participants had a varying number of months for which self-reported diarrhoea was present, the number of months for which self-report data was present was used as an offset in a negative binomial regression for rates. All models were estimated using GEE with an exchangeable correlation structure and robust standard errors. In adjusted models, the cluster proportion with carer reported diarrhoea at baseline was included as a covariate. Participants without any data were excluded. A further measure was carer-reported diarrhoea at end of follow-up. This was modelled using a logistic regression as above; including the cluster proportion with caregiver reported diarrhoea at baseline as a covariate in adjusted models.

*Role of the funding source*

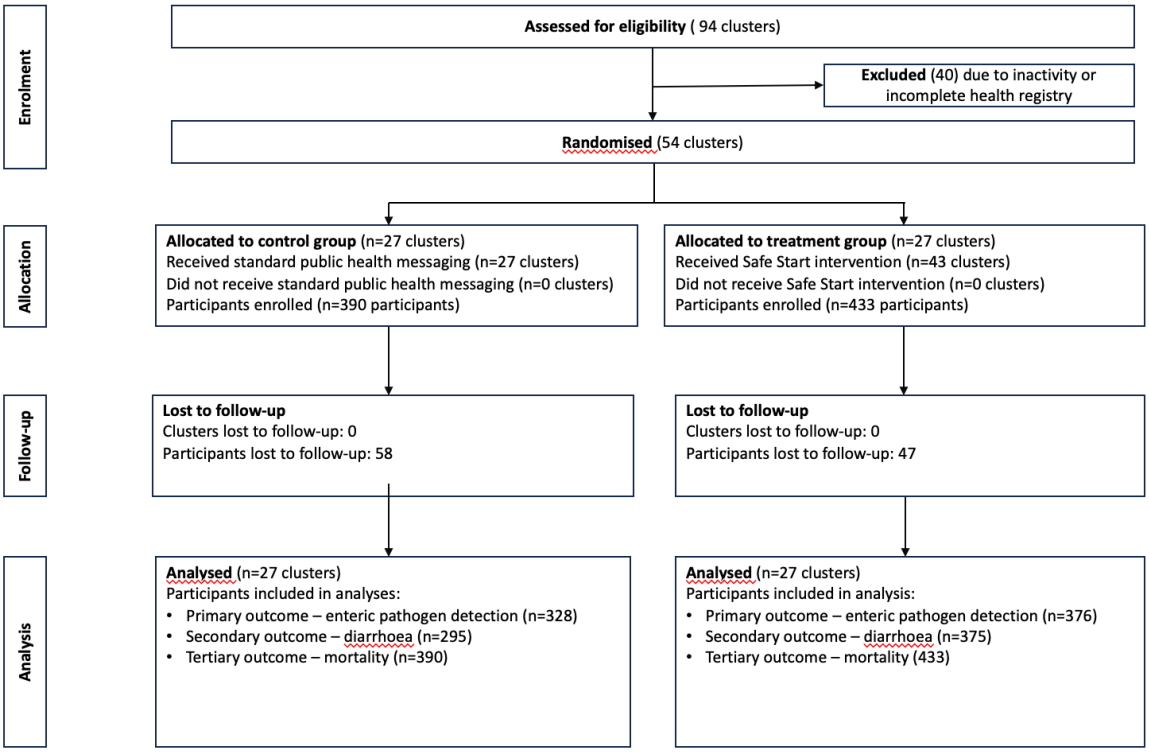
The funder had no role in the execution of the study nor the analysis of study data. The corresponding authors had full access to all the data in the study and had the final responsibility for the decision to submit for publication.

## Results

*Descriptive*

Eight hundred and eighty-eight infants were screened for study eligibility of which, 823 (390 in the control and 433 in the intervention) were enrolled in the study between March 26th 2018 and January 11th 2019, across 54 clusters (CONSORT diagram; Figure 7.1). Infant, caregiver, household infant feeding practices, water, sanitation and hygiene, and health outcome characteristics were well-balanced across the control and intervention arms at baseline (Table 7.1). The one-week period prevalence of diarrhoea was very similar in the two arms at baseline; 15.1% (59/390) in the control arm and 14.8 (64/433) in the intervention arm. The detection prevalence for one or more pathogens and the mean number of pathogens detected per infant were also very similar in the two arms at baseline; in the control arm, 87.8% (330/390) of children were positive for at least one pathogen, and the mean number of pathogens was 2.27; and in the intervention arm the corresponding figures were 90.0% (376/433) and 2.29.

Figure 7.1: CONSORT diagram




Almost all caregivers reported that the infant had ever been breastfed (99.8%), and 99.5% (388/390) and 99.3% (430/433) of participants in the control and interventions arms respectively were currently breastfed at baseline (22 weeks of age). At baseline, only 4.9% (19/390) in and 5.3% (23/430) of caregivers in the control and intervention arms respectively reported that their infant had been fed solid food in the last day. The mean number of children per household was the same in both arms (1.4) and households in the control and intervention arms appeared balanced in terms of economic status with similar proportions using electricity as their primary lighting source, owning a functioning refrigerator, and owning animals.

Access to water, sanitation and hygiene was well balanced in the two arms (Table 7.1). Almost all (99%) households in both arms had access to an improved water source, and 57.7% (225/390) and 56.4% (244/433) of households in the control and intervention arm reported treating their drinking water. A similar proportion of households in the two arms

(control: 84.0%; intervention: 88.2%) had access to an improved latrine but most households shared their sanitation facility with other households, and this was near equal across arms (89.5% and 89.6%). Soap was only observed the handwashing location in 6.9% (27/390) and 6.7% (29/433) of households in the control and intervention arms respectively.

Table 7.1: Baseline characteristics of participants in control and intervention groups

| | **Total** | **Control** | **Intervention** |
|---|---|---|---|
| | % (n) | % (n) | % (n) |
| **Infant** | | | |
| Total enrolled participants | 100 (823) | 47.4 (390) | 52.6 (433) |
| Participant female | 50.7 (417) | 49.0 (191) | 52.2 (226) |
| Rotavirus vaccination completed * | 97.5 (749) | 98.3 (353) | 96.8 (396) |
| Participant ever breastfed | 99.8 (821) | 100.0 (390) | 99.5 (431) |
| Participant currently breastfed | 99.6 (818) | 99.5 (388) | 99.3 (430) |
| Participant ever fed non-breastmilk milk | 28.9 (238) | 30.5 (119) | 27.5 (119) |
| Participant fed solid food in last day | 5.1 (42) | 4.9 (19) | 5.3 (23) |
| **Caregiver** | | | |
| Caregiver marital status is single | 12.0 (99) | 10.0 (39) | 13.9 (60) |
| Caregiver highest education level is primary | 39.3 (323) | 38.7 (151) | 39.7 (172) |

| | | | |
|---|---|---|---|
| **Household** | | | |
| Number of children in household (mean, n) | 1.4 (823) | 1.39 (390) | 1.4 (433) |
| Primary energy source for lighting is electricity | 91.1 (750) | 92.8 (362) | 89.6 (388) |
| Household has a functioning refrigerator | 14.6 (120) | 15.9 (62) | 13.4 (120) |
| Household owns any animals | 13.5 (111) | 12.8 (50) | 14.1 (61) |
| **Water, sanitation and hygiene access** | | | |
| Household has access to an improved latrine | 86.3 (710) | 84.0 (328) | 88.2 (382) |
| Sanitation shared with other households | 89.6 (737) | 89.5 (349) | 89.6 (388) |
| Household has access to improved water source | 99.0 (814) | 99.0 (386) | 99.0 (428) |
| Household treats drinking water | 57.0 (469) | 57.7 (225) | 56.4 (244) |
| Soap observed at handwashing location | 6.8 (56) | 6.9 (27) | 6.7 (29) |
| **Outcomes** | | | |
| Participant had diarrhoea in previous week | 15 (123) | 15.1 (59) | 14.8 (64) |
| Participant positive for one or more pathogens | 88.9 (706) | 87.8 (330) | 90.0 (376) |
| Enteric pathogens per participant (mean, n) | 2.27 (794) | 2.29 (376) | 2.25 (418) |

* Denominator is the 768 participants for whom an immunization card was available

** Denominator is 794 as stool samples could not be obtained from 29 participants

*Food hygiene behaviours and infant food contamination*

Caregivers were asked at baseline and at endline whether they had washed their hands in the previous 24 hours and, if so, when they had washed their hands. The results of this (Table 7.2) suggest small differences between the intervention and control arms for the behaviours associated with food hygiene but no difference for handwashing after defecation which was similarly high between groups at baseline and at subsequent timepoints. Although the prevalence of the food-related hand hygiene behaviours – handwashing before preparing food and before feeding food to the infant – were consistently higher in the intervention arm, the differences were small and increased in both arms between baseline and endline (Table 7.2). The food samples were collected at 32 weeks of age (± 1 week) samples and the proportion contaminated (>0 Enterococcus CFU) was lower in the intervention (34.4%) than the control arm (37.0%) but we found no evidence for a true difference between arms (adjusted odds ratio: 0.89, 95%CI 0.69 – 1.17; Table 7.2). Figure 7.2 is a Box and Whiskers plot of the Enterococcus concentrations in food samples by trial arm; showing very similar distributions.

Table 7.2: Prevalence of targeted handwashing behaviours before and after the intervention by trial arm, and prevalence of non-contaminated infant food at midline by trial arm

| | | **Pre-intervention** | **Post-Intervention*** ** |
|---|---|---|---|
| | | % (n) | % (n) |
| **Handwashing after defecation*** | | | |
| | Control | 89.4 (345/386) | 91.1 (308/338) |
| | Intervention | 91.3 (388/425) | 91.4 (361/395) |
| **Handwashing before feeding*** | | | |
| | Control | 56.2 (217/386) | 73.4 (248/338) |
| | Intervention | 54.8 (233/425) | 77.7 (307/395) |
| **Handwashing before preparing food*** | | | |

| | | | |
|---|---|---|---|
| | Control | 58.8 (227/386) | 72.8 (246/338) |
| | Intervention | 62.6 (266/425) | 77.5 (306/395) |
| **Food contaminated**** | | | |
| | Control | - | 37.0 (125/337) |
| | Intervention | - | 34.4 (135/392) |

* Caregiver reported behaviours

** >0 Colony Forming Units (CFU) of Enterococcus per gram of food

*** Post-intervention handwashing was assessed at 37 weeks of age (+/- 1 week) at study exit, and food contamination was assessed at 32 weeks of age (+/- 1 week)

*Enteric pathogen detection*

Stool samples were collected from 794 (96%) and 704 (85%) infants at baseline and endline respectively. At baseline, the control and intervention arms appeared well balanced for the primary outcome of enteric pathogen detection of one or more of the 23 pathogen-associated genes (Table 7.1). The pathogen detection prevalence was 88.9% (330/376) and 87.8% (376/418) in the control and intervention groups respectively, at baseline. At endline the prevalence of pathogen detection was 97.3% in the control and 96% in the intervention with no evidence for a difference between arms (adjusted odds ratio: 0.80, 95%CI: 0.33-1.95; Table 3). Arms were similarly well-balanced at baseline for the median number of pathogens detected per infant stool sample (Table 7.1) and there was no difference at endline (rate ratio: 0.99; 95%CIs: 0.92-1.07).

Table 7.3: Intervention effects on the pathogen detection prevalence and longitudinal prevalence of diarrhoea among infants

| **Outcome** | **Control group** | **Intervention group** | **Crude effect** | **p-value** | **Adjusted effect** | **p-value** |
|---|---|---|---|---|---|---|
| **Primary outcome** | % (n) | % (n) | Odds ratio (95% CI) | | Odds ratio* (95% CI) | |
| Enteric pathogen detection prevalence | 97.3% (319/328) | 96.5% (363/376) | 0.79 (0.32, 1.93) | 0.61 | 0.80 (0.33, 1.95) | 0.65 |
| **Secondary outcome** | Median (IQR), n | Median (IQR), n | Rate ratio (95% CI) | | Rate ratio** (95% CI) | |
| Diarrhoea longitudinal prevalence | 3 (0, 9), 295 | 1 (0, 3), 375 | 0.31 (0.23, 0.41) | <0.001 | 0.30 (0.23, 0.40) | <0.001 |

*Adjusted for the mean baseline pathogen prevalence by cluster.

**Adjusted for the proportion with carer reported diarrhoea by cluster.

*Diarrhoeal disease*

Longitudinal diarrhoea was measured through calendars provided to caregivers at two points in the study follow-up (25 and 32 weeks of age) and they were asked to mark days when the child had diarrhoea. For the 823 infants enrolled in the study, 151 (18%) of caregivers did not receive the calendars so these children were excluded from the analysis. A total of 674/823 (82%) of households were issued with calendars but the research team was only able to retrieve the first calendar from 619/674 households and the second calendar from 362/674 households resulting in missing data for many participants. The longitudinal prevalence of diarrhoea was approximately 70% lower in

the intervention arm than the control arm (Rate Ratio (RR): 0.30; 95%CIs: 0.23, 0.41). A sensitivity analysis was carried out using multiple imputation to account for the missing data. The total number of days with diarrhoea were imputed using the total number of detected pathogens measured at baseline and end of study as well as intervention and cluster. Multiple imputations by chained equations were used with predictive mean matching for all variables (intervention and cluster which had no missing values) and clustering was accounted for by using cluster in the imputation model. The results when imputed values were included for missing data were similar. The one-week period prevalence of diarrhoea at endline (37 weeks of age) was lower in the intervention arm (14.5%) than the control arm (10.9%) but we found no evidence for a true difference (aOR: 0.75; 95% CIs: 0.45, 1.26) (Table 7.4).

Table 7.4 : Effects on number of pathogens detected and the diarrhoeal disease prevalence at midline and endline

| | **Control group** | **Intervention group** | **Crude effect** | **p-value** | **Adjusted effect** | **p-value** |
|---|---|---|---|---|---|---|
| **Outcomes** | % (n) | % (n) | Odds ratio (95% CI) | | Odds ratio* (95% CI) | |
| Caregiver-reported diarrhoea in previous week | 14.5 (48/332) | 10.9 (42/386) | 0.77 (0.45, 1.31) | 0.33 | 0.75 (0.45, 1.26) | 0.28 |
| *Enterococcus* (>0 CFU) detected in infant food sample | 37,0 (125/337) | 34,4 (135/392) | 0.89 (0.68, 1.17) | 0.43 | 0.89 (0.68, 1.17) | 0.43 |
| | Median (IQR), n | Median (IQR), n | Rate ratio (95% CI) | | Rate ratio** (95% CI) | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Number of enteric pathogens detected | 3 (2,5), 328 | 3 (2,5), 376 | 0.99 (0.92, 1.06) | 0.73 | 0.99 (0.92, 1.07) | 0.77 |

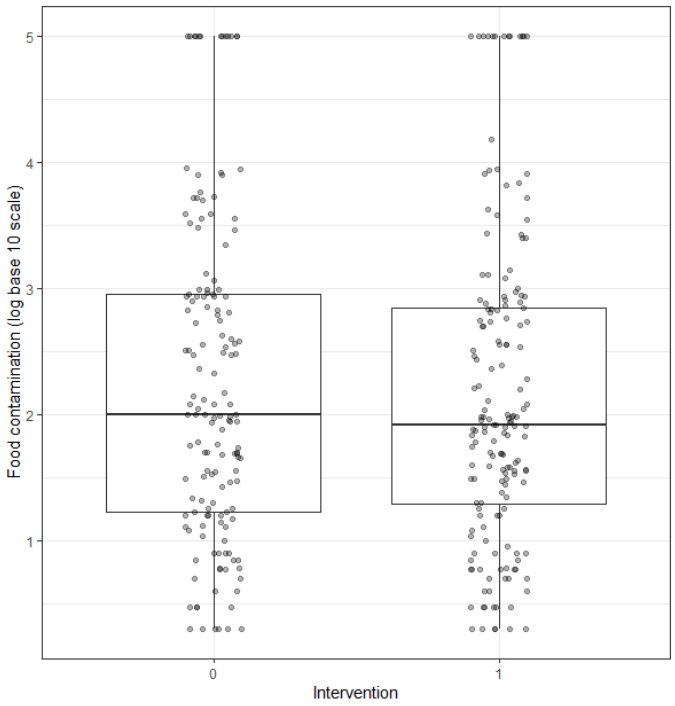
* Adjusted for the proportion with carer reported diarrhoea by cluster

** Adjusted for the mean baseline pathogen count by cluster

*Mortality*

There were no deaths reported during the study in either arm.

Figure 7.2: Box and whisker plots of *Enterococcus* concentrations in food samples by trial arm



## Discussion

In this trial we assessed the effect of a food hygiene intervention delivered through the existing health system on enteric pathogen detection and diarrhoea among infants residing in informal urban neighbourhoods of Kisumu Kenya. The intervention had no effect on our primary outcome of the prevalence of one or more enteric pathogens in infant stool at 37 weeks of age (adjusted odds ratio: 0.80; 95%CIs: 0.33-1.95). We found that the longitudinal prevalence of diarrhoea was lower in the intervention arm (adjusted rate ratio: 0.81; 95% CIs: 0.23-0.4) but the large amount of missing data meant these results could be an artifact. To our knowledge this is the first trial of food hygiene intervention targeting infants in low-income informal urban and evaluating the effect on the simultaneous detection of a broad range of enteric pathogens in stool.

There have been two previous comparable trials to assess the effectiveness food hygiene interventions on child health, one in the Gambia (8) and one in Malawi (17). These trials did not assess effects on enteric pathogen detection but both reported significant reductions in diarrhoea as reported by caregivers. While the interventions were similar to the intervention evaluated here – seeking to change caregiver food hygiene behaviours to reduce exposure to foodborne health risks – there are important differences. First, these interventions were of far higher intensity. In the Gambia trial (8), the intervention similarly featured interactive sessions led by community health volunteers, but these were delivered weekly for six months. And, in the Malawi trial (17), the authors report different interactive activities being delivered multiple times per month and over several months (31 weeks). Second, both these trials were implemented in rural areas which are very different to the urban informal neighbourhoods of Kisumu. Complex urban environments pose particular challenges due to the combination of high population density and inadequate water and sanitation infrastructure lead to highly contaminated environments. Third, the interventions in these two previous trials targeted a broader age interval spanning infancy and early childhood; 6-24 months of age in the Gambia, and between 1 – 24 months of age in Malawi. Although we emphasise caution in interpreting our reported effect on diarrhoea due to the significant missing data, our results are broadly consistent with those of the two previous trials (8, 17).

To our knowledge, this is the first trial of an infant food hygiene intervention with enteric pathogen detection as the primary outcome. Using molecular assays such as the TaqMan Array Card which permit the simultaneous detection of numerous pathogen-associated gene targets offer significant advantages over the main alternatives (27). The two previous analogous trials discussed above (8, 17) both used caregiver-reported diarrhoea which dominates the epidemiologic literature concerning environmental health interventions to prevent faecal-oral transmission of diarrhoeal diseases (28). The risk of bias in trials that combine subjective outcome measures, such as self-reported diarrhoea, with non-blinded study designs, where participants and research teams are aware of the treatment allocation, is self-evident but this has been demonstrated empirically across a range of interventions and health outcomes (29).

The intervention was developed through formative research addressing food contamination (20), caregiver practices (18), community health volunteer experiences (30), and delivered through existing public health structures, but the intervention failed to reduce contamination of infant food. There are various contextual factors that may explain this. First, the intervention occurred in a complex urban setting with high population density, limited access to safe drinking water and sanitation services, as well as other environmental risks, including prevalent animal ownership; all of which contrive to produce high exposure risks as reported in previous studies in Kisumu (31). In such a setting, where there are so many environmental hazards, and uncontained human and animal waste, this intervention may simply have been insufficient to prevent contamination of food. Second, beyond the immediate risks of environmental contamination posed by these hazards, the insufficient water supply may have limited caregivers' ability to practice handwashing around food preparation and feeding as promoted under the intervention. Households in these neighbourhoods have limited access to safely managed water supplies, and approximately 45% rely on water kiosks with the cost of water limiting consumption (32). In a previously published mixed methods process evaluation of this intervention, caregivers reported that whilst the handwashing container facilitated handwashing water scarcity acted as a barrier to their

use (33). A recent systematic review for the effect of handwashing with soap on child mortality found that these interventions were effective only when implemented in settings where sufficient water was available (34). Lastly, these informal settlements are highly dynamic; housing is relatively cheap, and residents often combine rural agricultural activities with urban income generation. As a result, caregiving is highly dynamic; a previous study into caregiving practices in the study areas found that over half of children had three or more routine caregivers within a given day (18). Whilst previous studies (7, 8, 35) of food hygiene interventions targeting a single caregiver, the mother, have been successful, these have exclusively been in rural areas with less dynamic caregiving structures than in this context.

The intervention was designed to target four critical control points to prevent foodborne health risks for infants: handwashing before food preparation and feeding, bringing the infant food to the boil before feeding, safe storage of all infant food, and use of designated feeding utensils. The two targeted handwashing behaviours - before preparing food and before feeding - increased in both arms by study exit (infant age: 37 weeks, +/- 1 week) but to a greater extent in the intervention (Table 7.2). This was consistent with previously reported results from structured observations of food hygiene practices at 32 weeks of age (33). That the reported behaviours increased in both arms suggests reporting bias associated with the interaction with community health workers which was intentionally balanced between arms through the active control design (23). The differences observed between arms in the targeted hand hygiene behaviours are much smaller than reported in previous trials (8, 35). Changes in the three other three areas were assessed through structured observation in a previously published process evaluation (33). Caregivers often failed to bring infant food to the boil before feeding and re-serving; likely due to the marginal costs – both financial and time – required to do this (33). Caregivers predominantly prepared food for the family – including the infant – early in the morning to save on fuel and then stored the cooked food in a flask. Caregivers were reluctant to use the sealed storage containers provided by the study team. According to caregivers, the containers were small in size and therefore not able to store enough food for the infant to last the entire day. Also,

caregivers complained that the containers were not able to keep the infant's food warm throughout the day.

## Limitations

The most important limitation of this study is that the retrieval rate for the calendars used to record diarrhoea was low and different between arms. We found a large reduction in diarrhoea using the available data which was similar when including imputed values for the missing data. Ultimately, though, these results may be an artifact. Furthermore, and again with regard to the diarrhoea results but also self-reported food hygiene behaviours, there is a clear risk of reporting bias given neither the caregivers nor the enumerators could be blinded to the intervention given its nature. We did though employ an active control arm which may have reduced bias associated with a greater intensity of interaction with agents promoting public health messages (eg the CHVs) in the treatment arm which is a common weakness in public health trials. The residual difference between arms – given that reported hygiene behaviours increased in both arms but to a greater extent in the intervention arm – was consistent with the estimates produced through separate structured observations at 32 weeks of age published previously (33).

## Conclusions

This intervention to improve food hygiene practices by infant caregivers delivered by trained agents working with Community Health Volunteers and through the existing public health structures had no effect on the detection prevalence of enteric pathogens among infants. We found that diarrhoea was 70% lower in the intervention group, but due to high and differential degree of missingness this may be an artifact. A reduction in diarrhoea of this magnitude achieved through an intervention designed with and delivered through existing public health structures would be important. We recommend further research to investigate whether similar interventions in similar complex urban

environments can reduce diarrhoeal disease at this critical stage as infants transition from breast-feeding to solid foods, and replicate the positive results from other trials in rural areas. However, this intervention failed to reduce the prevalence of enteric pathogen detection and larger scale, more comprehensive interventions are likely required to mitigate the myriad environmental hazards in complex urban settings such as these.

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# 8

# Chapter 8: Effectiveness of integrating water treatment and hygiene promotion into outpatient treatment of severe acute malnutrition among children in Senegal

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| **Student ID Number** | 279050 | **Title** | Mr |
| **First Name(s)** | Oliver | | |
| **Surname/Family Name** | Cumming | | |
| **Thesis Title** | The influence of water, sanitation and hygiene conditions on enteric pathogen exposure among vulnerable children – evidence from two trials | | |
| **Primary Supervisor** | Prof. Tanya Marchant | | |

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## Abstract

### Background

Severe acute malnutrition (SAM) affects approximately 17 million children globally. The aim of this study was to assess the effectiveness of integrating a household water treatment and hygiene promotion intervention within the national protocol of Senegal improved SAM recovery and other related outcomes.

### Methods

This was a cluster-randomized controlled trial with clusters corresponding to health centres allocated 1:1 to receive either the standard protocol alone or the standard protocol with the addition of the water treatment and hygiene intervention. The primary outcome of the trial was the SAM recovery rate as defined under the national in Senegal, and the secondary outcomes were weight gain, referral to inpatient care, diarrhoea, and all-cause mortality.

### Findings

2411 children were enrolled in the study of which 832 abandoned the outpatient programme before discharge. There was no difference in the proportion of children recovering from SAM at eight weeks post admission (39.6% vs 39.7%; aOR 0.95, 95% CIs 0.66, 1.45). There was no difference in weight gain, referral, mortality or enteric pathogen detection between groups. The prevalence of diarrhoea at eight weeks follow-up was higher in in the control group (20.0%) than the intervention group (12.5%), with evidence for a large difference between arms, accounting for between-group differences at baseline (aOR: 0.36; 95%CIs: 0.26, 0.50).

### Interpretation

The integration of household water treatment and hygiene promotion in the standard national protocol did not improve recovery nor the related outcomes of weight gain, referral, enteric pathogen detection or mortality but did reduce diarrhoea. Our results suggest that the addition of a WASH kit to the standard protocol would not improve

SAM outcomes in this setting but would potentially reduce the burden of diarrhoea among this vulnerable group.

Funding

This study was supported by the Bureau of Humanitarian Assistance of the United States Agency for International Development.

## Introduction

Severe acute malnutrition (SAM) - or severe wasting and/or nutritional oedema - results from inadequate nutrient intake and recurrent illnesses and persists as an issue of global health concern. In children SAM is defined as a weight-for-height z-score (WHZ) that is more than three standard deviations below the median of WHO child growth reference standards and/or nutritional oedema (1). This condition leads to increased susceptibility to infectious diseases, such as diarrhoea and pneumonia, and a high risk of mortality (2). In 2022, 45 million children were affected by acute malnutrition globally of whom 14 million experienced severe acute malnutrition (SAM), and of these only one third are estimated to have received appropriate treatment (3).

In its recently updated guidelines, the World Health Organisation (WHO) continues to recommend the Community-based Management of Acute Malnutrition (CMAM) model for treatment of uncomplicated SAM cases (1). Under this approach, which has been adopted by numerous governments and international agencies, children are treated at home with regular visits to outpatient facilities (1). The CMAM approach is regarded as a successful innovation that has reduced costs to both the health system and the individual, and improved the cost-effectiveness of treatment (4). Despite this success, recovery, referral and relapse rates for CMAM programmes vary greatly between settings, and often fall short of the global standard of 75% recovery (5). Identifying the causes of this variability and developing strategies to mitigate poor treatment outcomes can contribute to more effective programmes.

One factor which may contribute to poor recovery outcomes for children treated through CMAM are environmental risks at the household level, such as unsafe drinking water and sanitation. Whilst the shift to an outpatient-based model of treatment offers many advantages and has demonstrated improved outcomes, moving from a more controlled hospital setting to generally less controlled household settings may increase the risk of infection for children at a time when they are particularly susceptible. Water sanitation and hygiene conditions at the household and community level have been

found to be associated with increased risk of SAM, a higher risk of infection during outpatient-treatment (6), longer duration of outpatient-treatment (6), and higher risk of relapse following discharge from outpatient-treatment (7). Furthermore, trials in Pakistan (8) and in Chad (9) reported that the integration of drinking water treatment alone and combined with soap and hygiene promotion respectively resulted in improved recovery outcomes when integrated into CMAM programmes.

In response to this emerging evidence, various international agencies, including WHO (10), UNICEF (11), Action Against Hunger (12) and the World Bank (13), have called for the integration of water, sanitation and hygiene intervention in strategies to prevent and manage malnutrition. In the Sahel region in particular, where this is a high burden of SAM, there have been efforts to integrate WASH interventions within CMAM programmes, and several international agencies have advocated for the provision of a "WASH kit" to children on admission to CMAM treatment programmes to reduce risk of infection and thereby support recovery and prevent complications requiring referral. A recent systematic review however concluded that the evidence for the effectiveness of integrating water, sanitation and hygiene inventions in out-patient treatment programmes was limited and that further high-quality studies are needed (14). The aim of the "Traitement Intégré de la Sous-Nutrition Aiguë" (TISA) trial was to assess whether the integration of a household water treatment and hygiene promotion intervention within the national CMAM protocol of Senegal improved SAM recovery and other related outcomes.

## Methods

*Study design and setting*

This study was a cluster-randomized controlled trial with health centres serving as clusters. The health centres have responsibility under the Ministry of Health's national protocol of management of acute malnutrition (Protocol National de la Prise en Charge de la Malnutrition [PECMA]) for managing uncomplicated SAM cases and referral of complicated cases for inpatient treatment in hospitals. These centres – or Unité de Récupération et d'Education Nutritionnelle ambulatoire (UREN) – are charged with the identification of SAM cases and subsequent outpatient treatment through weekly visits to the UREN until discharge.

The trial was conducted across four districts (Podor, Pété, Linguère, and Dahra) of two departments (Podor and Linguère) in northern Senegal with a total population of approximately 600,000. This region has poor access to basic services (health, education, water and sanitation) and subsistence is based on agriculture or nomadic livestock practices, with traditional labor-intensive practices still prevalent. When the study site was selected, the prevalence of SAM in the two departments was 1.1% (0.6-2.0%) in Podor and 1.8% (0.3-9.5%) in Linguere (Ref), and approximately 50,000 people were estimated to be at risk of food crisis during the lean season.

The trial was pre-registered with the ClinicalTrials.gov public registry on 16 December 2020 (NCT04667767). The study protocol was approved the Comité National d'Ethique pour la Recherche en Santé de Sénégal (000179/MSAS/DPRS/CNERS) and the Research Ethics Committee of the London School of Hygiene and Tropical Medicine in the United Kingdom (17511).

*Participants*

All uncomplicated SAM cases aged 6-59 months diagnosed in the included clinics were eligible for participation. Inclusion criteria for the study correspond to the diagnostic criteria for SAM used in Senegal: weight-for-height z-score <-3; or brachial perimeter

(mid-upper arm circumference) <115; or bilateral oedema. Patients meeting the diagnostic criteria for SAM but who also had medical complications are referred to inpatient care and were therefore excluded. Eligibility was assessed by the attending nurse who explained the study to the accompanying caregiver and invited them to participate. All eligible children for whom the parent provided informed consent and who did not meet the exclusion criteria were enrolled.

Nurses at participating health facilities assessed eligibility for participation and written consent was obtained by the senior nurse for the facility (ICP; Infirmier Chef de Poste). The designated nurse at each health facility explained the study to the caregiver using a standard Participant Information Sheet and Consent Statement that was read in Wolof or Pular according to preference. Hard copies of both documents were provided to all participant caregivers in Wolof or Pular. Only eligible children for whom the parent signed and dated a consent form were enrolled in the study. In the case of illiterate participants, an independent witness signed and dated the consent form and the participant drew a cross mark in the space provided. Caregivers were informed that they could withdraw from the study subsequent to enrolment, and any data already collected and analysed was used, unless the participant requested otherwise, but no further analysis was done nor samples kept. No incentives to participate were provided; however, any travel costs to the UREN that would not otherwise be incurred were reimbursed.

*Randomisation and masking*

Under the cRCT design, clusters representing the catchment areas for primary health care facilities (UREN) were allocated on a one-to-one basis to either a control group receiving the standard outpatient treatment programme (OTP) as per the national protocol for Senegal or an intervention group receiving the OTP plus the household water treatment and hygiene promotion intervention. Clusters were randomly allocated by a statistician at the London School of Hygiene and Tropical Medicine (LSHTM) using a random number generator. This was a public health intervention seeking to change specific behaviours through direct engagement with participants such that blinding of

participants to their allocation was not possible. Randomisation was conducted remotely but the data collection team, study investigators, and trial statistician were blinded to allocation. The trial statistician conducted the final analyses blinded to allocation.

*Procedures*

Participants in both the control and intervention groups received the national protocol for outpatient treatment for uncomplicated SAM cases ("la prise en charge de la malnutrition aigüe" [PECMA]). The PECMA is aligned with the WHO guidelines management of acute malnutrition(1). Here we describe briefly the standard national protocol for treatment of uncomplicated SAM and then the water treatment and hygiene promotion intervention, the integration of which is the focus of this trial.

The control group received the standard PECMA protocol comprising three broad elements. First, medical treatment, whereby patients receive weekly visits at the UREN for anthropometric assessment and appetite testing, antibiotic and anthelminthic treatment, and administration of vitamin A. Second, nutrition treatment whereby the caretaker weekly receives Ready-to-Use Therapeutic Food (RUTF) for the patient based on the weight of the child (measured weekly) together with instruction on how to prepare, store, and administer the RUTF to the child. Third, infant and young child feeding (IYCF) guidance through advice provided weekly and adapted to the individual child and caregiver.

The intervention group received a water treatment and hygiene promotion intervention integrated with the standard PECMA protocol. This intervention was designed to improve the quality of drinking water consumed by the child and to improve hand hygiene practices around the child whilst they received treatment for SAM. On admission the caregiver was provided with a kit comprising a sealable 20 litre (L) vessel with tap along with a weekly supply of sodium dichloroisocyanurate tablets (Medentech, Wexford, Ireland) sufficient for the daily treatment of 20 L/day for a week, and each week for the course of the child's treatment. The caregiver was instructed on

how to safely treat, store and dispense drinking water for the child by the attendant nurse at the point of admission. Two bars of soap were also provided on admission, and the attending nurse instruction on safe hygiene practices around the child including handwashing after defecation, before preparing food for the child and before feeding the child and/or eating. The intervention was further reinforced during the weekly visits of the caregiver and child to the clinic by the attending nurse as well as two visits to the household during the period of treatment by a community health worker ("Relais Communautaire").

*Outcomes*

The primary outcome of the trial was SAM recovery rate within eight weeks of admission to outpatient treatment for SAM. Recovery was defined as per the national protocol of Senegal: two consecutive measures at weekly health centre visits with WHZ ≥ -1.5, if admitted based on WHZ, and/or MUAC ≥ 125 mm, if admitted based on MUAC, and no oedema. The MUAC and WHZ score, as recorded in the health registry, was used to determine the criteria for admission (i.e., MUAC, WHZ, or both) and recovery was determined based on MUAC (the value recorded in the health registry) and/or the calculated WHZ score using weight and height values recorded in the health registry and computed based on WHO references.

An additional five secondary outcomes were all assessed in the eight weeks subsequent to admission to outpatient treatment for SAM. First, weight gain, defined as grams of weight gained by child per kilo per day between admission and exit. Second, the rate of referral, defined as the number of participants referred to the next level of clinical care. Third, the one-week prevalence of diarrhoea (three or more loose or liquid stools passed within 24 hours (WHO)) at eight weeks (+/-one week) post-admission as reported by participant caregiver. Fourth, all-cause mortality includes all deaths of participants recorded at the health centre during follow-up due to any cause. And fifth, the detection prevalence of one or more enteric pathogens at eight weeks (+/- one week) post-admission.

Health registry data was recorded by attending nurses at admission and all subsequent weeks of treatment through recovery and discharge, abandonment, referral, death, and then entered into a database using ODK forms. Data entry was verified by a remote team using anonymised images of registries to extract, enter and check data for consistency. Data was collected by the research team at admission.

At study exit, nurses collected a rectal swab from participants. Flocked nylon rectal swabs were eluted in 1 mL of liquid Amies solution (eSwab, Copan Diagnostics, catalogue #484CE). Approximately 40 µL of eluate was added to each of four spots on an FTA micro elute card (Qiagen, catalogue # WB120410) and air dried for a minimum of three hours before storage in individual plastic bags with desiccant. Samples were stored in the dark at ambient temperature until shipment to the London School of Hygiene & Tropical Medicine for extraction and molecular analysis. We analysed rectal swabs for 30 enteric pathogens using a microfluidic qPCR array card (TaqMan Array Card); the laboratory methods have been described previously by our team (15).

*Statistical analysis*

All statistical analyses were carried out in Stata, version 18 (16). For the original sample size calculation, we used the Hayes-Bennet formula for cRCTs (17). Assuming an intra-class correlation co-efficient of 0.09, and a recovery rate of 67%, based on historical data from the study area, we estimated that with 80% power, and an Alpha error of 5% a sample size of 1,720 children across 86 clusters would be sufficient for a minimum detectable difference (MDD) in the proportion recovered rates between arms. This MDD was judged appropriate based on the findings of an earlier trial of a very similar intervention (9).

Analysis for all primary and all secondary outcomes carried out at the individual level with adjustment for clustering within health centres. We adopted an “intention to treat” approach whereby data were analysed according to their allocation to either the intervention or control group, irrespective of participant response to the intervention.

Child's age and gender were adjusted for a priori, and further individual-level variables adjusted for if they appeared imbalanced between the groups.

For the primary outcome of recovery, the counts and proportions of children recovering in each arm are presented, and the odds ratio for recovery in the intervention group relative to the control group with 95% confidence intervals estimated using a mixed effects logistic regression model with random effects at the health centre level to account for clustering. For weight gain, a mixed effects linear regression model was used to estimate mean differences between groups. For mortality, referral and presence of enteric pathogens, counts and proportions of children in each arm will be presented, and odds ratios with 95% confidence intervals will be estimated using mixed effects logistic regression models. For the prevalence of diarrhoea, a mixed effects logistic regression model for the prevalence of diarrhoea at four and eight weeks post admission adjusted for differences between arms in the prevalence of diarrhoea at admission.

*Role of funding source*

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

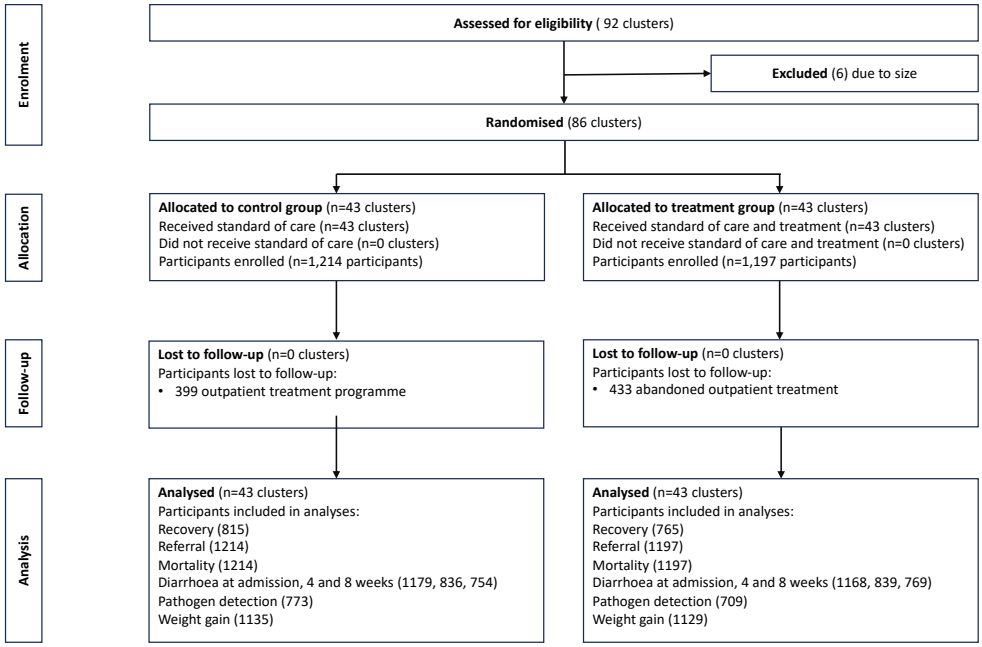
## Results

*Enrolment*

The start of our trial was delayed due to the COVID-19 pandemic and enrolment prolonged due to a series of external factors that have been previously described but notably included flooding and national strike action by health workers which affected facilities in the study area (18). Between 22 December 2021 and 20 February 2023, 2,411 children diagnosed with uncomplicated severe acute malnutrition (SAM) and admitted to outpatient treatment were enrolled in the study (Figure 8.2). The monthly

rate of enrolment was similar between arms.  1214 and 1197 participants were enrolled in the 43 control and 43 treatment clusters, respectively. Of the enrolled participants, 835 (35%) participants – comprising 400 (33%) in the control group and 435 (36%) in the intervention group - abandoned the outpatient programme during treatment before discharge, referral or death so did not complete the study and were considered as loss to follow-up.

Figure 8.1: CONSORT diagram for the TISA trial




*Descriptive*

A greater proportion of participants were female overall (40.0%) and within both the control (39.4%) and intervention (40.7%) arms (Table 8.1). The mean age of participants at admission to CMAM was 18.2 months and very similar in both arms. Overall, 63.0% of participants were currently breastfed at the time of admission, and this was very similar across arms. Most caregivers were female (94.4%), with a slightly lower proportion in the intervention (92.7%) than the control (96.1%) arm, and a slightly higher proportion of caregivers had not completed primary education in the intervention (73.5%) than the control (69.7%) arm. At admission, the health status of participants

was generally less favourable in the intervention arm compared to the control arm. This was most pronounced for diarrhoea with 34.3% of caregivers in the intervention arm reporting diarrhoea in the previous week compared to 20.8% in the control arm. The intervention group had consistently worse anthropometric indicators of undernutrition: worse mean weight-for-height z-scores (WAZ) [-3.23 versus -3.18], worse mean height-for-age z-score (HAZ) [-3.29 versus -3.25], worse mid-upper arm circumference (MUAC) [117.4 versus 118.7].

Table 8.1: Baseline characteristics in control and intervention groups

| | **Total** | **Control** | **Intervention** |
|---|---|---|---|
| | % (n) | % (n) | % (n) |
| **Participant data at admission*** | | | |
| Enrolled participants | 100.0 (2,411) | 50.4 (1,214) | 49.7 (1,197) |
| Participant female | 40.0 (2,411) | 39.4 (478) | 40.7 (487) |
| Mean age (months) | 18.2 (2,400) | 18.3 (1,211) | 18.1 (1,189) |
| Participant currently breastfed | 63.0 (1,293/2,051) | 62.8 (639/1,017) | 63.3 (654/1,034) |
| Mean weight-for-height Z-score** | -3.20 (2,411) | -3.18 (1,214) | -3.23 (1,197) |
| Mean mid-upper-arm-circumference | 118.1 (2,400) | 118.7 (1,205) | 117.4 (1,195) |
| Oedema present at admission | 0.3 | 0.3 | 0.4 |

| | | | |
|---|---|---|---|
| | (8/2,411) | (3/1,214) | (5/1,197) |
| Mean height-for-age Z-score* | -3.27 (2,411) | -3.25 (1,214) | -3.29 (1,197) |
| Diarrhoea in previous week | 27.5 (2,401) | 20.8 (251/1,209) | 34.3 (409/1,192) |
| Temperature ≥ 38 °C | 8.5 (204/2,411) | 7.2 (87/1,214) | 9.8 (117/1,197) |
| Participant received amoxicillin | 98.9 (2,226/2,252) | 98.8 (1,102/1,116) | 98.9 (1,124/1,136) |
| Participant received vitamin A | 80.2 (1,153/1,437) | 76.4 (490/641) | 83.3 (663/796) |
| Participant received mebendazole | 67.0 (778/1,161) | 67.6 (392/580) | 66.4 (386/581) |
| **Household level data at household data** | | | |
| Caregiver is female | 94.4 (2,224/2,356) | 96.1 (1,138/2,356) | 92.7 (1,086/2,356) |
| Caregiver not completed primary education | 71.6 (1,624/2,268) | 69.7 (793/1,138) | 73.5 (831/1,130) |
| **Water, sanitation and hygiene access** | | | |
| Household has improved drinking water source | 92.9 (2,188/2,335) | 94.2 (1,116/1,185) | 91.6 (1,072/1,170) |
| Household has an improved sanitation facility | 61.5 (1,442/2,344) | 66.5 (780/1,173) | 56.5 (662/1,171) |

| | | | |
|---|---|---|---|
| Household practices open defecation | 26.8 (629/2,344) | 18.9 (222/1,173) | 34.8 (407/1,171) |
| Household treats child's drinking water | 46.2 (1,078/2,336) | 46.4 (541/1,167) | 45.9 (537/1,169) |
| Household chlorinates child's drinking water | 18.4 (432/2,344) | 19.4 (226/1,167) | 17.6 (206/1,169) |
| Caregiver washes hands after defecation | 65.0 (1,466/2,257) | 66.2 (751/1,135) | 63.7 (715/1,122) |
| Caregiver washes hands before feeding child | 54.0 (1,219/2,257) | 58.5 (664/1,135) | 49.5 (555/1,122) |

Water, sanitation and hygiene conditions varied between arms (Table 8.1) with somewhat worse conditions in the intervention arm compared to the control arm for some aspects. A lower proportion of participant households in the intervention group had access to an improved sanitation facility (56.5%) and a higher proportion reported open defecation (34.8%) than in the control arm (66.5% and 18.9%, respectively). Reported caregiver handwashing practices were generally lower in the intervention arms versus the control arm but this was most pronounced for handwashing before feeding (49.5% versus 58.5%). Access to an improved drinking water source was greater than 90% in both arms but a little higher in the control arm (94.2% versus 91.6%) and reported treatment of drinking water was similar across arms.

*Intervention delivery*

In the intervention arm, 96.8% of the intervention arm received the WASH kit, and 88.4% received it as intended at admission to CMAM treatment with the remainder receiving it in a subsequent visit to the health facility (Table 8.2). A very small number (5/1214; <1%) of participants in the control arm received a WASH kit during the study which was likely due to confusion within the health system supply chain in the early stages of the trial. Households in both arms were visited by the research team at

approximately four weeks post-intervention to assess the presence of the WASH kit as well as reported behaviours around water treatment and handwashing. In 97.4% of the households visited in the intervention arm, the WASH kit container was being used to store drinking water consumed by the participant, compared to <1% in the control arm. And, the stored drinking water was being treated in 82.6% of households in the intervention arm compared to 8.2% in the control arm. More caregivers in the intervention arm (94.7%) than the control arm (87.0%) reported using soap for handwashing versus using water alone. The proportion of caregivers reporting handwashing after defecation was similar in the two arms but handwashing before feeding was higher in the intervention arm (63.1%) than in the control arm (53.7%).

Table 8.2: Intervention fidelity and response

| | **Total** | **Control** | **Intervention** |
|---|---|---|---|
| **Intervention fidelity and response** | % (n) | % (n) | % (n) |
| WASH kit provided during study* | 48.5 (1147/2369) | 0.4 (5/1,214) | 96.8 (1,142/1,180) |
| WASH kit provided at admission as planned** | 44.1 (1,063/2,411) | 0.4 (5/1,214) | 88.4 (1,058/1,197) |
| Child drinking water stored in TISA container | 49.01 (820/1673) | 0.7 (6/837) | 97.4 (814/836) |
| Stored drinking water is treated at household | 45.4 (761/1,677) | 8.2 (69/839) | 82.6 (692/838) |
| Caregiver uses soap for handwashing | 90.9 (1508/1659) | 87.0 (717/824) | 94.7 (791/835) |
| Caregiver washes hands after defecation | 79.5 (1,254/1,577) | 78.7 (612/778) | 80.4 (642/799) |

| | | | |
|---|---|---|---|
| Caregiver washes hand before feeding child | 58.4 (921/1,576) | 53.7 (417/777) | 63.1 (503/799) |

* The caregivers of participants in the control group were offered the WASH kit at study exit only

** WASH kit was provided on the day of admission as per protocol

*Primary and secondary outcomes*

For the primary outcome, ascertained through WHZ and/or MUAC values recorded in health registries, the proportion of children recovering from SAM was similar in the control (41.8%) and intervention groups (41.9%) and we found no evidence for a difference between arms (adjusted odds ratio [aOR] 0.95, 95% confidence intervals [CIs] 0.66 – 1.39) (Table 8.3). Recovery as recorded by nurses was higher in the intervention arm (58.2%) than the control arm (52.9%) but with no evidence for a difference (aOR: 1.08; 95%CIs: 0.72-1.62) (Table 8.3).

Table 8.3: Adjusted and crude effects for the primary and secondary trial outcomes

| | Control | | Intervention | | Crude effect | | | Adjusted[1] effect | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | n/N | % | n/N | % | Odds ratio | 95% CI | p-value | Odds ratio | 95% CI | p-value |
| **Primary outcome** | | | | | | | | | | |
| Recovery | 320/815 | 39.62 | 303/765 | 39.66 | 0.97 | 0.65,1.44 | 0.878 | 0.98 | 0.66, 1.45 | 0.908 |
| **Secondary outcomes** | | | | | | | | | | |
| Rate of referral | 16/1214 | 1.32 | 13/1197 | 1.09 | 0.79 | 0.30, 2.07 | 0.634 | 0.75 | 0.29, 1.99 | 0.569 |
| All-cause mortality | 5/1214 | 0.41 | 3/1197 | 0.25 | 0.59 | 0.13, 2.75 | 0.501 | 0.59 | 0.12, 2.90 | 0.512 |
| Diarrhoea prevalence | | | | | | | | | | |
| Week 0 | 374/1179 | 31.7 | 516/1168 | 44.2 | 1.00 | - | <0.001 | 1.00 | | <0.001 |
| Week 4 | 266/836 | 31.8 | 246/839 | 29.3 | 0.57 | 0.44, 0.75 | | 0.57 | 0.43, 0.75 | |
| Week 8 | 151/754 | 20.0 | 96/769 | 12.5 | 0.36 | 0.26, 0.50 | | 0.36 | 0.26, 0.50 | |
| Pathogen detection | 488/782 | 62.40 | 455/724 | 62.85 | 1.16 | 0.82, 1.56 | 0.397 | 1.15 | 0.79, 1.65 | 0.470 |
| | Mean | SE | Mean | SE | Difference | 95% CI | p-value | Difference | 95% CI | p-value |
| Weight gain, g/kg/d | 4.06 (N = 1135) | 0.12 | 4.38 (N=1129) | 0.17 | 0.24 | -0.40, 0.88 | 0.466 | 0.18 | -0.46, 0.82 | 0.578 |

[1] Adjusted for age, sex, diarrhoea and HAZ at admission, and household sanitation, water source

Weight gain was greater in the intervention arm (4.42 g/kg/d) than the control arm (4.10 grams per kg per day [g/kg/d]) with no evidence of a difference between arms (difference in means 0.25; 95%CIs: -0.40 - 0.89). Fewer participants were referred in the intervention arm (1.3%) in the intervention arm than the control arm (1.5%) with no evidence of a difference between groups (aOR: 0.78; 95%CIs: 0.31 – 2.00). Five deaths occurred in the control arm [0.3%]) and three in the intervention arm (0.3%) with no evidence for a difference between arms (aOR: 0.74%, 95%CIs: 0.13 – 4.20) (Table 8.3).

The prevalence of caregiver-reported diarrhoea at eight weeks follow-up was higher in in the control group (20.0%) than the intervention group (12.5%), with evidence for a difference between arms, accounting for between-group differences at admission (aOR: 0.36; 95%CIs: 0.26 – 0.50) (Table 8.3). There was also an effect albeit smaller at four weeks post-admission (aOR: 0.36; 95%CIs: 0.26 – 0.50). Caregiver diarrhoea was

also measured at the same time points, and we found similar reductions although the prevalence was much lower than among children.

The enteric pathogen detection prevalence at eight weeks follow-up was very similar in the two arms, with 63.0% and 64.0% of participants positive for at least one of the assessed enteric pathogens in the control and interventions arms respectively (Table 8.4). There was no evidence for a difference (aOR: 1.15; 95%CIs: 0.79 – 1.65) in the secondary outcome of enteric pathogen detection prevalence (Table 8.3).

Table 8.4: Enteric pathogen detection by arm

| **Pathogens** | **Control** N (%) | **Intervention** N (%) |
|---|---|---|
| **Total samples** | 773 (100.0) | 709 (100.0) |
| **One or more pathogens detected** | 448 (63.0) | 455 (64.0) |
| **Viruses** | | |
| Adenovirus (serotypes 40 & 41) | 6 (0.8) | 5 (0.7) |
| Astrovirus | 0 (0.0) | 1 (0.1) |
| Norovirus (genotypes I & II) | 2 (0.3) | 2 (0.3) |
| Rotavirus | 17 (2.2) | 17 (2.4) |
| Sapovirus | 0 (0) | 0 (0.0) |
| **Bacteria** | | |
| Enteroaggregrative *E. coli* (EAEC) | 253 (33.0) | 237 (33.0) |
| Shigatoxin producing *E. coli* (STEC) | 18 (2.3) | 15 (2.1) |
| *E. coli* O157 | 5 (0.6) | 3 (0.4) |
| Enteropathogenic *E. coli* (EPEC) | 157 (20) | 151 (21.0) |
| Enterotoxigenic *E. coli* (ETEC) | 76 (9.8) | 68 (9.6) |
| Enteroinvasive *E. coli* (EIEC) | 53 (6.9) | 65 (9.2) |
| *Shigella sonnei* | 5 (0.6) | 3 (0.4) |
| *Shigella flexneri* | 13 (1.7) | 20 (2.8) |
| Campylobacter_jejuni_coli | 35 (4.5) | 36 (5.1) |

| | | |
|---|---|---|
| *Salmonella enterica* | 6 (0.8) | 7 (1.0) |
| Salmonella typhi | 0 (0) | 1 (0.1) |
| Toxigenic *Vibrio cholerae* | 0 (0) | 0 (0.0) |
| *Clostridium difficile* | 15 (1.9) | 13 (1.8) |
| *Yersinia enterocolitica* | 1 (0.1) | 2 (0.3) |
| *Aeromonas* spp. | 9 (1.2) | 8 (1.1) |
| *Helicobacter pylori* | 0 (0.0) | 0 (0) |
| *Plesiomonas shigelloides* | 5 (0.6) | 4 (0.6) |
| **Protozoa** | | |
| *Cryptosporidium* spp. | 15 (1.9) | 9 (1.3) |
| *Giardia* spp. | 120 (16.0) | 88 (12) |
| *Entamoeba histolytica* | 2 (0.3) | 1 (0.1) |
| *Cyclospora cayetanensis* | 6 (0.8) | 3 (0.4) |
| **Helminths** | | |
| *Ascaris lumbricoides* | 1 (0.1) | 0 (0.0) |
| *Trichuris trichuria* | 2 (0.3) | 0 (0.0) |
| Hookworm | 0 (0.0) | 0 (0.0) |
| *Strongyloides stercoralis* | 3 (0.4) | 1 (0.1) |
| *Schistosoma* | 0 (0.0) | 2 (0.6) |

## Discussion

In this trial we assessed the effectiveness of integrating drinking water treatment and hand hygiene promotion into the national protocol for outpatient treatment of SAM in Senegal. We sought to assess the effectiveness of such an intervention when delivered at scale and through the existing structure of the health system. The intervention was successfully delivered at scale with almost 90% of participants in the intervention arm receiving the WASH kit on the day of admission into the existing outpatient treatment programme (CMAM). Furthermore, in household visits approximately one month later, almost all (97%) households were using the designated container for drinking water storage and over 80% were treating their water as recommended. We found no

difference though in the proportion of children recovering from SAM in the group receiving the water treatment and hygiene intervention in addition to the standard protocol. And, there was no difference in the related outcomes of weight gain during treatment, referral to hospital care nor all-cause mortality. However, diarrhoea was significantly reduced in the group receiving the water treatment and hygiene intervention at four weeks and eight weeks of follow-up.

There have been two previous trials to assess the effectiveness of integrating drinking water treatment and/or improved hand hygiene in CMAM programmes. The settings for these two trials – the Kanem region of Chad (9), and the Sindh province of Pakistan (8) - are quite different to ours. Whilst there is a high burden of acute malnutrition in northern Senegal, it is a stable setting with relatively high health system coverage, and a well-established protocol for community-based management of acute malnutrition (PECMA) that is administered directly by the government of Senegal. By contrast, the trials in Chad and Pakistan took place in settings with generally lower levels of access to health services where the CMAM programmes were delivered directly by external humanitarian agencies rather than by the Ministry of Health as in our study. Access to safe water and sanitation services in the study populations for these trials appeared worse than in our study suggested higher risk of environmental exposure to enteric pathogens. In the Pakistan trial, access to improved drinking water and sanitation ranged from 82.3-94.1% and 30-42% respectively across the study arms (8), compared to 92.9% and 61% in our trial. The trial in Chad (9) did not report on water and sanitation services but a separate case-control study (19) by the same team in the same population reported that 46.5% of the population practiced open defecation and 23.9% used unimproved drinking water sources which was much higher than in our population. Possibly linked to these underlying factors, admission criteria (WHZ, MUAC, presence of oedema) were consistently worse among participants in these two other trials compared to our own.

Our main finding - that the integration of a water treatment and hygiene promotion intervention into the standard national protocol for outpatient treatment of uncomplicated SAM did not improve recovery outcomes differs from these two earlier

trials of similar interventions (8, 9). Both of these previous studies reported significant increases in the proportion of children recovering from SAM among those receiving an integrated drinking water treatment and/or hygiene. A trial in Chad adopted a similar design to ours – a cluster-randomised controlled trial with allocation by clinic – to evaluate a similar intervention – the integration of a "WASH kit" into a standard CMAM approach - and found an absolute difference of 10.5% in the proportion recovering (95%CIs: 6.7, 19.8) in group receiving the WASH kit (9). A second trial in Pakistan described as a, "site-randomised trial" evaluated the effectiveness of integrating three different drinking water treatment interventions, chlorine tablets, a double-action flocculant/disinfectant product, and ceramic filters. All three of these water treatment interventions were found to be associated with increased recovery rates compared to a control site receiving standard CMAM and with chlorine tablets, as included in our intervention, associated with the greatest odds of recovery (aOR: 2.5; 95%CIs:1.7, 3.9). There are three important differences in how recovery was assessed in these trials compared to ours. First, we assessed recovery at eight weeks post-admission compared to 12 weeks in the Chad trial and 17 weeks in the Pakistan trial. We selected eight weeks on the basis that the SPHERE guidelines recommend… and because the probability of abandonment was known to be high post eight weeks. Given that the proportion recovered is cumulative it is likely that the proportion recovered in our trial would have been greater if follow-up had been longer, but we cannot know whether this would have resulted in a difference between arms. Second, the criteria for recovery differed in these trials compared to ours. In Chad, if a patient was admitted on WHZ (<-3.0), recovery required the patient to achieve a WHZ of ≥-2.0 compared to ≥ -1.5 in our study. In Pakistan, patients were assessed for recovery on MUAC (≥125mm) only. Third, the nature, dosage and intensity of these interventions differed to ours.

We found a large effect on the prevalence of diarrhoeal disease at eight weeks post-admission (aOR: 0.36; 95%CIs: 0.26 – 0.50) and at four weeks post-admission (aOR: 0.57; 95%CIs: 0.26, 0.50). This effect estimate accounts for the higher prevalence of diarrhoea at admission in the intervention group compared to the control group. Interestingly, we found an effect among caregivers too which is coherent given that the intervention focused on caregiver hand hygiene before preparing food and feeding the

child which would plausibly confer protection on the caregiver as well as the participant. Furthermore, it also seems plausible that the caregiver consumed treated water from the container given that the caregiver was again responsible for treatment and storage, and given that sufficient chlorination tablets were provided for 20 L per day, which would permit consumption by both individuals. By comparison, the trial in Chad found no effect on diarrhoea but the trial in Pakistan found that chlorine tablets – the same type as used in our trial – but not the other water treatment interventions (double-action disinfectant/flocculant or ceramic filter) were associated with a lower risk of diarrhoea. Notably, the prevalence of diarrhoea at admission was lower in Chad than in Pakistan or in our study.

Our trial included enteric pathogen detection as a secondary outcome but found no effect on the prevalence of detecting one or more pathogens in participants' stool. There are several advantages of using stool-based enteric pathogen detection via multiplex methods in trials of water, sanitation and hygiene(20). Notably they provide an objective measure of exposure to a given pathogen that lies on the causal pathway between environmental hazards and disease outcomes. In our trial, we incorporated these methods to enhance our understanding of if - and how - the intervention modified participants' exposure to environmental hazards. A recent meta-analysis found that WASH interventions were only associated with small reductions in enteric pathogen detection in the environment but on basic sanitation interventions were included (21), and not water treatment or hand hygiene interventions such as was evaluated in our trial. A separate individual participant analysis assessed the association between enteric pathogen detection in the environment (soil, children's hands, and stored drinking water) and subsequent detection in children, childhood diarrhoea and growth faltering (22). Interestingly, environmental detection was associated with increased risk of detection in children and lower HAZ but not diarrhoea (22). There have been few WASH trials incorporating enteric pathogen detection as outcomes and, to our knowledge, this is the first WASH trial to do so in the context of SAM treatment and recovery. Whilst not among SAM outpatients, one trial of a water treatment technology that included enteric pathogen detection as an outcome reported no effect but this was in a community setting (23). Future analysis of our data at the individual pathogen level

will allow us to assess the effect of the intervention and to assess the relationships between environmental factors, enteric pathogen detection, diarrhoea and nutritional status.

Our trial was designed to assess the effectiveness of integrating this water and hygiene intervention within existing policy and systems and at a scale that would provide evidence for deployment at a national level. The trial was successfully implemented through 86 health centres, over two years of enrolment, and across 25,000 $km^2$ which accounts for over 10% of the land mass of Senegal. Given the scale – and the challenges encountered during enrolment that included the COVID-19 pandemic, severe flooding, and health system strikes (18) – it is notable that the intervention was successfully delivered by the health system with almost 90% of participants receiving the intervention on the day of admission as intended. Furthermore, when assessed at the household level approximately four weeks post-admission almost 100% of households in the intervention group were using the container, and over 80% treating the water in the container as intended. Handwashing with soap – versus water alone – was higher in the intervention group as was handwashing before feeding the child as was intended under the intervention. A sub-study of the trial that included 445 participant households, confirmed this picture, finding both higher residual chlorine levels and reduced microbial contamination in the intervention group compared to the control group (Braun et al 2025). A major question that informed our trial and the underlying theory of change was whether such an intervention could feasibly be integrated within national policy and systems – as opposed to being delivered by external agents at a more limited scale of a project or programme within a discrete area or population as in previous studies. Our results suggest that this approach is feasible although the costs and benefits would need to be carefully considered in any given setting.

## Limitations

Despite the random allocation of the treatment by cluster, there appeared to be imbalance between arms for certain covariates. At baseline, the intervention group had a lower proportion of participants that had access to an improved sanitation facility (56.5% versus 66.5%) and a lower proportion of caregivers reported washing their hands before their child (49.5% versus 58.5%) compared to the control group. There was no difference however for the proportion with access to an improved drinking water source or who reported treating their drinking water. In addition, the one-week period prevalence of diarrhoea was higher in the intervention group compared to the control group at admission (34.3% versus 20.8%). Whilst our analyses were adjusted for these covariates on the basis of this observed imbalance. We took a decision to not use a balancing method in our randomization on the basis of the large number of clusters enrolled but those designing future trials might consider this. The previous trial in Chad randomized in stratified pairs according to monthly admission numbers to balance enrolment across arms but still had significant imbalance for diarrhoea on admission. A recent analysis has demonstrated that random allocation within geographically matched pairs improved statistically efficiency across all considered outcomes, including outcomes relevant to our trial (24). Such an approach would have been feasible for our trial and may have yielded greater balance and statistical efficiency.

We sought to assess the real-world effectiveness – and feasibility – of this intervention when integrated into existing health policy and systems. Such trials can be termed as "pragmatic trials" and are generally motivated by concerns as to the value for policy of trial results where efficacy of interventions has been optimized (25). Our trial revealed underlying challenges that may be less apparent in more controlled trial settings or indeed in humanitarian settings were populations are bound to limited geographies due to security and/or availability of shelter and services. The rate of abandonment we observed was very high but similar to the official figures and likely reflects challenges for households in balancing the benefits of continuing treatment versus the financial and time costs for accessing treatment when facilities are distant and the relative cost

of transport high. A further contributing factor is the “transhumance” - or nomadic pastoralism whereby families move with their animals to seek pasture during dry seasons - which makes accessing and sustaining treatment challenging (26). Our trial was designed to estimate effect of integrating this intervention within the existing health systems and as such our results account for these factors by design, and provide evidence that can inform investment decisions in this setting. The high rate of abandonment suggests that interventions or targeted approaches to tackle the high rate of abandonment before recovery may yield improved outcomes.

## Conclusions

The integration of household water treatment and hygiene promotion to the standard treatment protocol did not improve recovery nor the related outcomes of weight gain and referral but did reduce diarrhoea. Our results suggest that in this setting, the addition of a WASH kit to the standard protocol would not improve SAM outcomes but would potentially reduce the burden of diarrhoea among this vulnerable group.

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# 9

## Chapter 9: General discussion and conclusions

The aim of this thesis was to assess the effectiveness of WASH interventions in reducing enteric pathogen exposure and improving related health consequences in two vulnerable populations. In this concluding chapter, I summarise the main findings related to each of the research objectives outlined in Chapter 1. I then go on to discuss the main limitations of this thesis and close with three recommendations each for current policy and future research in this area.

## Main findings

Objective 1: Review current evidence in relation to enteric pathogen exposure in childhood and its consequences, and discuss the implications for water, sanitation and hygiene interventions

This objective was addressed in the Background section of the thesis under Chapters 2 and 3. In the published review that forms Chapter 2, I reviewed the evidence for the effect of WASH interventions on stunting and how WASH interventions could be delivered to optimise their potential contribution to reducing the global burden of childhood stunting. Chapter 3 built on the findings of Chapter 2 but focused specifically on enteric pathogen exposure and the implications for WASH interventions.

The main findings from Chapter 2 were that there are multiple social and biological pathways through which access to WASH services can negatively affect childhood undernutrition and contribute to stunting. The biological pathways are mediated through environmental exposure to enteric pathogens leading to diarrhoea, intestinal worm infections, and environmental enteric dysfunction; all of which are strongly associated with growth faltering and other developmental consequences. The social pathways have been less well researched but are numerous and important. These include the calorific cost of water carriage when water sources are distant, the effects of stress related to water and sanitation insecurity when services are unsafe, and the direct financial cost of water on the food budget or the indirect opportunity cost when productive time is spent transporting and/or queuing for water. It is clear however that WASH alone will not eliminate stunting, but it does have the potential to accelerate progress as part of more comprehensive and targeted strategies. In high-risk settings where there is little or no access to safely managed services and the burden of enteric disease and childhood undernutrition is high, the challenge is what can be done to limit the risk of enteric pathogen exposure among the most vulnerable groups and when they are most susceptible.

Chapter 3 focused on enteric pathogen exposure and its measurement via stool-based pathogen detections using multiplex PCR methods. There were four main findings. First, that chronic environmental exposure to enteric pathogens has far-reaching effects on the healthy growth and development of children, and these effects can be independent of diarrhoeal disease. Second, the results of observational and interventional studies of limited or basic WASH interventions have found little or no effect on enteric pathogen exposure among children. It is clear that higher levels of WASH service will be required to prevent exposure but there is a paucity of epidemiological evidence to confirm whether safely managed water and sanitation services combined with basic hygiene are sufficient. Third, there are settings and populations from whom safely managed services are decades away based on current rates of progress, and in these settings sustained exposure to enteric pathogens presents significant health risks. And, lastly, there have been no rigorous studies of interventions to prevent exposure among vulnerable groups at high-risk times that have measured changes in exposure via stool-based enteric pathogen detection. These findings informed the focus of the research to assess interventions specifically designed to reduce exposure in two high-risk settings during two critical moments, weaning and treatment for severe acute malnutrition. And, to measure changes in exposure directly using multiplex PCR.

Objective 2: Assess the burden of enteric pathogen detection prevalence in two different vulnerable populations

Chapters 6 and 8 addressed this objective and provided estimates for the enteric pathogen detection in two different vulnerable populations. The two estimates are not directly comparable due to the study design. In Kenya as reported in Chapter 6, the detection prevalence of enteric pathogens was assessed at baseline in both groups before the intervention, as well as after the intervention. In Senegal, due to resource constraints, pathogen detection was assessed only at endline, at eight weeks post-admission to SAM treatment. The prevalence in the control arm which did not receive the water and hygiene intervention offers a reasonable point of comparison. In both settings most children had one or more enteric pathogens detected in their stool.

The detection prevalence was higher at baseline in Kenya (89.3% positive for one or more pathogens) compared to the prevalence among children in Senegal at study exist at eight weeks post-admission (64% positive for one or more pathogens). As the control group in Senegal were regularly visiting the health post and being treated for SAM, including receiving ready-to-use therapeutic food (RUTF) that would reduce foodborne exposure to pathogens, the lower prevalence may reflect a protective effect of these non-WASH aspects. The main finding is that in both vulnerable groups there is a high degree of environmental exposure to a range of pathogens as evidenced by the high rates of detection in stool. The risk this presents to these two groups – weaning infants of less than six months of age, and children diagnosed with severe acute malnutrition – is high, and the results support the rationale for targeted interventions to reduce exposure at this time.

Objective 3: Evaluate the effect of an infant food hygiene intervention on enteric pathogen detection and related health outcomes in a complex urban environment

Objective 3 is addressed by the results reported in Chapters 6 and 7. The main result was the infant food hygiene intervention had no effect on enteric pathogen detection and therefore seemingly failed to reduce exposure. The trial also assessed the effect on diarrhoeal disease and found a large difference between arms. Although robust to a sensitivity analysis using imputed values, the high and differential degree of missingness in the diarrhoea data means these results may be an artifact. The main finding therefore is that this targeted intervention failed to reduce enteric pathogen exposure among this vulnerable group as intended, and almost all infants in both arms were positive for at least one pathogen (97.3% and 96.5% in the control and intervention arms respectively). This degree of exposure to enteric pathogens among young infants poses significant immediate and long-term health risks.

Objective 4: Evaluate the effect of a water treatment and hand hygiene intervention on enteric pathogen detection and related health outcomes among children with severe acute malnutrition

Objective 4 was addressed by the results from the TISA trial reported in Chapter 8. The main finding was the intervention had mixed effects on the assessed outcomes. The integration of household water treatment and hygiene promotion to the standard treatment protocol did not reduce enteric pathogen detection nor improve SAM recovery but did reduce diarrhoea. This intervention failed to reduce enteric pathogen exposure and possibly as result failed to improve SAM recovery or the related outcomes of weight gain and referral. These results therefore do not support the integration of this intervention within outpatient treatment for SAM as a means of improving recovery rates.

Interestingly, though, the intervention had a large effect on diarrhoea among the participants and also on diarrhoea among the participants' caregivers who were ultimately targeted by the intervention. These results are at risk of reporting bias but the consistency and coherence with changes in practices in terms of water treatment and handwashing lend weight to the findings. If these results are valid, there are two important conclusions. The first is that, as suggested by the results from previous studies (1, 2), the primary mechanism linking poor environmental conditions and resultant enteric pathogen exposure to growth and developmental outcomes may not be diarrhoea. Instead, it is the sustained and underlying exposure to enteric pathogens – as measured through stool-based detection – that may explain this. The implication of this is that WASH interventions that fail to reduce enteric pathogen detection in children are unlikely to offer growth and developmental benefits. The second implication though is that whilst the integration of this intervention to the standard protocol would not improve SAM outcomes it could potentially reduce the burden of diarrhoea among this vulnerable group.

## General conclusions and recommendations

The thesis addressed its aim of assessing the effectiveness of WASH interventions in reducing enteric pathogen exposure and improving related health consequences in two vulnerable populations. And, the main findings have been summarised above in relation to the four subordinate objectives. There are several limitations to this research, but these have been discussed within each of the three results chapters (Chapters 6-8). There are two major

limitations which warrant restating here though before drawing conclusions or making recommendations.  First, the diarrhoea results from the trial in Kenya are problematic. The limitations of these data prohibit interpreting the observed differences as evidence of an effect but, conversely, they do not provide confirmatory evidence for a null effect. The second limitation is the high rate of abandonment within the national programme for outpatient treatment of SAM in Senegal. Arguably a strength of the TISA trial was that it was designed as a pragmatic trial to assess the effect of integrating this intervention with the national programme as it exists. However, this limits inference as to the *potential* effect of such an intervention in settings where a greater proportion of children complete treatment as recommended by the WHO.

To conclude this thesis, I make a limited set of recommendations for policy and research based on the findings of the research that has been presented and discussed.

## Implications for policy

I make three broad areas of policy recommendation.

Firstly, these results lend further weight to the ambition of the Sustainable Development Goal of universal access to safely managed water and sanitation services, and basic hygiene facilities. The rationale for these interventions was there are settings where universal access to safely managed services is a distant prospect but where there is high enteric pathogen exposure that presents significant risks for vulnerable children. The interventions were designed with the health system with the aim of delivering targeted interventions that might reduce enteric pathogen exposure among vulnerable groups at critical times, such as weaning or outpatient treatment for SAM. These interventions failed to reduce exposure and therefore the recommendation is for long-term planning and investment to extend access to safely managed services to all. These interventions were designed and delivered by the health sector – and motivated by health sector concerns around child health and undernutrition - but delivering safely managed services sits with other sectors and ministries.

Secondly, these studies draw attention to specific vulnerabilities of certain groups in high-exposure environments. The high prevalence of enteric pathogen prevalence in these two groups – young weaning infants and children with severe acute malnutrition – is a major public health concern. And this enteric pathogen exposure results from a lack of infrastructure and services that can ensure that a sufficient quantity of drinking water of good quality is available to households, and that human waste is effectively managed and does not enter the environment untreated. Undernutrition - both chronic and acute – is multifactorial and reflects multiple deprivations, including poverty, food insecurity, lack of access to high quality health services, and poor WASH services. The recommendation here is for greater coordination between the sector/s and ministries responsible for WASH services, and the health system in order to identify and target investment at settings and populations at greatest risk.

Lastly, there is a need too for pragmatism. There are settings and populations where reliable access to safely managed water and sanitation services, and basic hygiene facilities is a distant prospect. At different scales and affecting different groups, this situation exists in many countries around the world. At current rates of progress, it might take decades of planning and investment to reach universal access to safely managed WASH services as envisioned under the SDG. In these settings, the recommendation is for incremental risk-based strategies to move vulnerable populations towards safely managed WASH services. How this is done will reflect the specific challenges of a given context but what is generalisable is the need for long-term strategy and planning that is geared towards incremental risk reduction.

### Implications for future research

Finally, I make a limited set of recommendations for future research in this area.

First, I recommend the further integration of clinical and environmental pathogen detection using multiplex PCR in WASH-related epidemiology and more broadly in other areas of environmental health. These methods address longstanding concerns in the field of WASH-

related epidemiology about the reliance on subjective outcome measures and resulting high risk of bias. These methods can strengthen observational and interventional WASH studies which have traditionally relied on weak methods to assess the key parameters of hazard, exposure, and disease. Practically, the information generated can confirm whether these interventions have succeeded in their primary purpose of preventing environmental exposure to pathogens.

Second, the questions raised by these research findings are not necessarily amenable to randomised intervention studies in the first instance. There is a need for more robust observational studies with longitudinal follow-up to understand the changing environmental risks through childhood in relation to enteric pathogen exposure and its consequences. There have been multiple important studies that have followed children in the first years of life and demonstrated the relationship between enteric pathogen exposure and growth and development. However, we lack studies that have at the same characterised the dynamic environment within which this happens. Studies that combine longitudinal assessment of enteric pathogen detection in children with longitudinal assessment of pathogens in their environments will strengthen out understand of when and how risks occur.

My last recommendation is a more personal reflection on the centrality of gender in how these health problems, and the related interventions and research, are understood. It was striking to me how in these two very different settings, how a gendered understanding of child health ran through the underlying health structures, the conception and delivery of the specific interventions, and indeed how the health consequences were understood. In essence, in both settings, the health of children was understood as the responsibility of the mother and by extension – whether implicitly or explicitly – a failure to protect children's health was understood as a failure of the mother. This was most striking in the health post registries of the TISA trial where abandonment or default were often recorded simply as, "négligence de mamon" (the negligence of the mother). A recent systematic re-review of WASH interventions which had featured in trials highlights the extent of this (3). Using the WHO Gender Responsiveness Assessment Scale, all 133 interventions included were classified as either gender unequal or gender unaware, indicating that all these WASH interventions were "exploitative". My recommendation would be that future public health research in this area

investigate how and why women are held responsible for such public health problems and what is the impact on these women of this.

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# APPENDICES

# Appendix 1

Appendix 1: Ethical Approvals for Safe Start trial

**GREAT LAKES UNIVERSITY OF KISUMU**
**(GLUK)**

P. O. Box: 2224-40100 KISUMU, Tel: 254-057-2023972,
Cell. 0712 054 623
*Email: ethicalreview@gluk.ac.ke*

**CERTIFICATE OF APPROVAL OF RESEARCH PROTOCOL**
**GLUK Research Ethics Committee (GREC)**
**Ref: No. GREC/010/248/2016**

| | |
|---|---|
| **Date of submission** | April 02, 2016 |
| **Study Protocol**: | A cluster randomized controlled trial (cRCT) |
| **Title**: | **THE EFFECT OF A NOVEL CHILDHOOD HYGIENE INTERVENTION ON ENTERIC INFECTIONS AND GROWTH FALTERING IN LOW-INCOME INFORMAL SETTLEMENTS IN KISUMU, KENYA** |

| | | |
|---|---|---|
| **Persons Submitting the Protocol:** | | |
| **Co Investigators** | **J. Mumma** | - Great Lakes University of Kisum, GLUK **(PI)** |
| | **D. Nelima** | - GLUK |
| | **J. Anderson** | - University of Florida |
| | **R. Dreibelbis** | - University of Oklahoma |
| | **E. Aseyo** | - GLUK |
| | **K. Baker** | - University of Iowa |
| | **Z. Mahmud** | - International Center Diarrhoeal Disease Research, Bangladesh |
| | **E. Allen** | - London School of Hygiene and Tropical Medicine (LHSTM) |
| | **D. Kaseje** | - GLUK |
| | **O. Cumming** | - LSHTM |

| | |
|---|---|
| **Approval Date**: | Friday, April 22, 2016 |
| **Approval Expiration Date**: | October, 2018 |
| **Type of Review**: | Minimum Quorum of Board |

We are glad to inform you that your application for local ethical review has been analyzed and approved on the basis of compliance with the Committee's satisfaction with the protocol's scientific validity, justification, relevance of purpose and assurance on the necessary ethical considerations and conditions required of a standard norm.

It is our belief that collaborating institutions in the study finds equal satisfaction in the protocol outline. Kindly submit a Dholuo and Swahili translated Informed Consent versions before commencement of the study. Otherwise, we wish you all the best in the study process. As collaborators, we would also request for copies of the ensuing reports for purposes of record as the investigation unfolds.

Always quote the GREC reference in future correspondence and all applications / re-submissions.

[redacted]

*Rev. Boniface Obondi*
**SECRETARY – GREC**

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**Observational / Interventions Research Ethics Committee**

Mr Oliver Cumming
Assistant Professor
Department of Disease Control (DCD)
LSHTM

1 February 2018

Dear Mr Oliver Cumming,

**Study Title:** Safe Start Trial - a Cluster Randomised Controlled Trial of a Infant Food Hygiene Intervention in Kisumu, Kenya

**LSHTM ethics ref:** 14695

Thank you for your application for the above research, which has now been considered by the Interventions Committee.

**Confirmation of ethical opinion**

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

**Conditions of the favourable opinion**

Approval is dependent on local ethical approval having been received, where relevant.

**Approved documents**

The final list of documents reviewed and approved by the Committee is as follows:

| Document Type | File Name | Date | Version |
|---|---|---|---|
| Investigator CV | Oliver Cumming CV | 22/12/2017 | 1 |
| Investigator CV | Robert Dreibelbis CV | 22/12/2017 | 1 |
| Investigator CV | Jane Mumma_CV | 22/12/2017 | 1 |
| Investigator CV | Kelly Baker_CV | 22/12/2017 | 1 |
| Other | GCP certifcate_Oliver Cumming | 22/12/2017 | 1 |
| Protocol / Proposal | Safe Start_QUESTIONNAIRES_(Cumming) | 22/12/2017 | 1 |
| Information Sheet | Safe Start PIS (Cumming)_control group | 22/12/2017 | 1 |
| Information Sheet | Safe Start PIS (Cumming)_intervention group | 22/12/2017 | 1 |
| Information Sheet | Safe Start_CONSENT FORM (Cumming) | 22/12/2017 | 1 |
| Sponsor Letter | GLUK Approval_Safe Start Trial | 22/12/2017 | 1 |
| Local Approval | GLUK Approval_Safe Start Trial | 22/12/2017 | 1 |

**After ethical review**

The Chief Investigator (CI) or delegate is responsible for informing the ethics committee of any subsequent changes to the application. These must be submitted to the Committee for review using an Amendment form. Amendments must not be initiated before receipt of written favourable opinion from the committee.

The CI or delegate is also required to notify the ethics committee of any protocol violations and/or Suspected Unexpected Serious Adverse Reactions (SUSARs) which occur during the project by submitting a Serious Adverse Event form.

An annual report should be submitted to the committee using an Annual Report form on the anniversary of the approval of the study during the lifetime of the study.

At the end of the study, the CI or delegate must notify the committee using an End of Study form.

All aforementioned forms are available on the ethics online applications website and can only be submitted to the committee via the website at: http://leo.lshtm.ac.uk

Additional information is available at: www.lshtm.ac.uk/ethics

Yours sincerely,

**Professor John DH Porter**
**Chair**

ethics@lshtm.ac.uk
http://www.lshtm.ac.uk/ethics/

**Improving health worldwide**

# Appendix 2

Appendix 2: Participant Information Sheet and Consent Form for Safe Start trial

**INFORMED CONSENT FORM**

Full Title of Project: Safe Start Trial

Name of Principal Investigators: Jane Mumma and Oliver Cumming

| | **Please initial box** |
|---|---|
| 1. I confirm that I have read and understand the participant information sheet dated XX.XX.XXXX for the above study. I have had the opportunity to consider the information, ask questions and have had these answered fully. | |
| 2. I understand that my participation is voluntary and I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. | |
| 3. I understand that the information I provide as part the study may be looked at by responsible individuals from the London School of Hygiene & Tropical Medicine and Great Lakes University of Kisumu and from regulatory authorities, where it is relevant to my taking part in this research. I give permission for these individuals to access my records. | |
| 4. I give permission to audio-record any conversations and understand the procedures that will be used to keep this information confidential | |
| 5. I agree to take part in the above study. | |

| Name of Participant *(printed)* | Signature/Thumbprint | Date |
|---|---|---|
| Name of Person taking consent | Signature | Date |

The participant is unable to sign. As a witness, I confirm that all the information about the study was given and the participant consented to taking part.

| Name of Impartial Witness *(if required)* | Signature | Date |
|---|---|---|

*1 copy for participant; 1 copy for Principal Investigator*

**Safe Start Trial: Participant Information Sheet**

Intervention Group

You are being invited to take part in a study by researchers from the Great Lakes University of Kisumu (GLUK) London School of Hygiene and Tropical Medicine (LSHTM), and the University of Iowa (UI). The study has been approved by the Ethics Committee of LSHTM, and the Institutional Review Boards of GLUK. We are implementing this research in collaboration with Kisumu Country Ministry of Health.

Before you decide whether to take part in this study, it is important for you to understand why the research is being done and what is involved if you decide to participate. Please ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. The following is to explain details of the study:

**1. What is the purpose of the study?**
The purpose of the study is to understand more about why young children become sick in your community, and to test an intervention to reduce this. By testing this intervention and collecting information about the health of your child we hope to find ways to improve public health in Kisumu.

**2. What is the intervention?**
The intervention is designed to help caregivers improve hygiene in the household and includes certain products and training that will be delivered by your Community Health Volunteer with support from a GLUK student. Half of those participating in the study will receive the intervention during the study and the other half will not receive the intervention.

**3. How do you decide who gets the intervention?**
At the beginning of the study, it was decided by lottery which CHVs would be in the group whose households would receive the intervention and which CHVs would be in the group whose households would not receive the intervention. Whether you get the intervention depends on which group *your* CHV was allocated to.

**4. Will I receive the intervention?**
You are in the group that will receive the intervention.

**5. Why have I been chosen?**
You are being asked to participate in this study as you live in the area selected for this study (Nyalenda). Within Nyalenda we expect 750 infants to participate in this study.

**6. Do I have to take part?**
It is up to you whether or not to participate in this study. We will describe the study and go through this information sheet. If you agree to take part, we will then ask you to sign two copies of the consent form. If you prefer, you may indicate your consent by making a thumbprint instead. Both copies will be signed by me, and one will be left with you and I will take one away for our records.

**7. What will happen if I don't want to participate in the study?**
You can decide to stop participating in the study at any time. If you withdraw from the study, your decision will be kept strictly confidential and, if you request this, all collected information will be destroyed.

**8. What will happen to me if I take part?**
If you agree to take part in this study, I will ask you to take part in the activities:

Today, if you agree to participate in the study, to answer some questions regarding your household, and regarding the living conditions in your household and compound, including questions on electricity, water and sanitation. This should take no more than 30 minutes.

Approximately three months from now we will visit you again and ask you to answer some questions and also to show us how prepare food and feed your infant. We also ask you to provide a small amount of the food you prepared for your infant. This should take no more than 45 minutes.

Approximately four months from now we will visit you again I will ask you to assist me in obtaining a stool sample from your child. To do this I will give you a sealable plastic container for the stool sample and a plastic bag to place the container in. I, or another member of the research team, will collect it tomorrow morning. If it was not possible to get a sample, we can come back the following morning. This should take no more than 30 minutes.

In addition to these visits, you will be visited regularly by your CHV who will check the health of your infant and provide information on what to do if they fall sick.

**9. What are the possible disadvantages and risks of taking part?**
Getting the stool sample from each child will not cause pain and will be done by you and not by a member of the research team.

**10. What are the possible benefits of taking part?**
We cannot promise the study will help you but the data we collect may help to improve the living conditions and public health in Kisumu by providing information on how available resources can best be used to improve people's health and quality of life.

**11. Will my taking part in the study be kept confidential?**
Yes: all information collected about you during the course of the research will be kept strictly confidential. The stool samples will be analysed after names have been removed from the samples.

**12. What will happen to the samples and the results of the research study?**

The food samples taken from your household will be analysed in a laboratory here in Kisumu at the Great Lakes University of Kisumu campus. The stool samples will be sent to the University of Iowa in the United States for analysis. The stool samples may be stored in a freezer to allow further analyses in the future.

All the data from the questionnaires and the samples will be analysed by researchers working with the London School of Hygiene and Tropical Medicine, University of Florida, Great Lakes University of Kisumu and the Kenya Medical Research Institute. Results of the questionnaires will be summarized anonymously and presented at community meetings convened by Great Lakes University Kisumu which you will be invited to and you will be able to ask questions of the research team if there is anything which you do not understand. The overall results of the research will be presented to the Kisumu County Ministry of Health and published in scientific journals.

**12. What if something goes wrong?**
If you have a concern about any aspect of this study, you should ask to speak to my supervisor who will try to answer your questions. If you would like to complain about any aspect of the study, please contact the lead investigators:

- Jane Mumma – GLUK (Kisumu, Kenya): +254 715709272
- Oliver Cumming –LSHTM (London, UK); +44 207 636 8636

**13. Who do I contact in an emergency?**
*The following contact is available 24 hours a day for the duration of the study:*

Sheillah Simiyu (GLUK): +254 722 215291

**You will be given a copy of the information sheet and a signed consent form to keep.**
**Thank you for considering taking the time to read this sheet.**

## Safe Start Trial: Participant Information Sheet

Control Group

You are being invited to take part in a study by researchers from the Great Lakes University of Kisumu (GLUK) London School of Hygiene and Tropical Medicine (LSHTM), and the University of Iowa (UI). The study has been approved by the Ethics Committee of LSHTM, and the Institutional Review Boards of GLUK. We are implementing this research in collaboration with Kisumu Country Ministry of Health.

Before you decide whether to take part in this study, it is important for you to understand why the research is being done and what is involved if you decide to participate. Please ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. The following is to explain details of the study:

**1. What is the purpose of the study?**
The purpose of the study is to understand more about why young children become sick in your community, and to test an intervention to reduce this. By testing this intervention and collecting information about the health of your child we hope to find ways to improve public health in Kisumu.

**2. What is the intervention?**
The intervention is designed to help caregivers improve hygiene in the household and includes certain products and training that will be delivered by your Community Health Volunteer with support from a GLUK student. Half of those participating in the study will receive the intervention during the study and the other half will not receive the intervention.

**3. How do you decide who gets the intervention?**
At the beginning of the study, it was decided by lottery which CHVs would be in the group whose households would receive the intervention and which CHVs would be in the group whose households would not receive the intervention. Whether you get the intervention depends on which group *your* CHV was allocated to.

**4. Will I get the intervention later?**
You are in the group that will not receive the intervention during the study but at the end of the study when your child is 37 weeks old we will provide you with the same products that households in the intervention group received.

**5. Why have I been chosen?**
You are being asked to participate in this study as you live in the area selected for this study (Nyalenda). Within Nyalenda we expect 750 infants to participate in this study.

**6. Do I have to take part?**
It is up to you whether or not to participate in this study. We will describe the study and go through this information sheet. If you agree to take part, we will then ask you to sign two copies of the consent form. If you prefer, you may indicate your consent by making a thumbprint instead. Both copies will be signed by me, and one will be left with you and I will take one away for our records.

**7. What will happen if I don't want to participate in the study?**
You can decide to stop participating in the study at any time. If you withdraw from the study, your decision will be kept strictly confidential and, if you request this, all collected information will be destroyed.

**8. What will happen to me if I take part?**
If you agree to take part in this study, I will ask you to take part in the activities:

Today, if you agree to participate in the study, to answer some questions regarding your household, and regarding the living conditions in your household and compound, including questions on electricity, water and sanitation. This should take no more than 30 minutes.

Approximately three months from now we will visit you again and ask you to answer some questions and also to show us how prepare food and feed your infant. We also ask you to provide a small amount of the food you prepared for your infant. This should take no more than 45 minutes.

Approximately four months from now we will visit you again I will ask you to assist me in obtaining a stool sample from your child. To do this I will give you a sealable plastic container for the stool sample and a plastic bag to place the container in. I, or another member of the research team, will collect it tomorrow morning. If it was not possible to get a sample, we can come back the following morning. This should take no more than 30 minutes.

In addition to these visits, you will be visited regularly by your CHV who will check the health of your infant and provide information on what to do if they fall sick.

**9. What are the possible disadvantages and risks of taking part?**
Getting the stool sample from each child will not cause pain and will be done by you and not by a member of the research team.

**10. What are the possible benefits of taking part?**
We cannot promise the study will help you but the data we collect may help to improve the living conditions and public health in Kisumu by providing information on how available resources can best be used to improve people's health and quality of life.

**11. Will my taking part in the study be kept confidential?**
Yes: all information collected about you during the course of the research will be kept strictly confidential. The stool samples will be analysed after names have been removed from the samples.

**12. What will happen to the samples and the results of the research study?**

The food samples taken from your household will be analysed in a laboratory here in Kisumu at the Great Lakes University of Kisumu campus. The stool samples will be sent to the University of Iowa in the United States for analysis. The stool samples may be stored in a freezer to allow further analyses in the future.

All the data from the questionnaires and the samples will be analysed by researchers working with the London School of Hygiene and Tropical Medicine, University of Florida, Great Lakes University of Kisumu and the Kenya Medical Research Institute. Results of the questionnaires will be summarized anonymously and presented at community meetings convened by Great Lakes University Kisumu which you will be invited to and you will be able to ask questions of the research team if there is anything which you do not understand. The overall results of the research will be presented to the Kisumu County Ministry of Health and published in scientific journals.

**12. What if something goes wrong?**
If you have a concern about any aspect of this study, you should ask to speak to my supervisor who will try to answer your questions. If you would like to complain about any aspect of the study, please contact the lead investigators:

- Jane Mumma – GLUK (Kisumu, Kenya): +254 715709272
- Oliver Cumming –LSHTM (London, UK); +44 207 636 8636

**13. Who do I contact in an emergency?**
*The following contact is available 24 hours a day for the duration of the study:*

Sheillah Simiyu (GLUK): +254 722 215291

**You will be given a copy of the information sheet and a signed consent form to keep.**
**Thank you for considering taking the time to read this sheet.**

# Appendix 3

Appendix 3: Ethical Approvals for TISA trial

REPUBLIQUE DU SENEGAL
Un Peuple – Un But – Une Foi

MINISTERE DE LA SANTE
ET DE L'ACTION SOCIALE

CNERS
SENEGAL
Comité National d'Ethique
pour la Recherche en Santé

N° 00000138 MSAS/DPRS/CNERS

Dakar, le 19 AUG 2019

La Présidente

# AVIS ETHIQUE ET SCIENTIFIQUE

***Référence :*** **Protocole SEN19/45 : « TISA-Un essai contrôle randomisé par grappes pour évaluer l'effet de l'ajout d'un traitement de l'eau à domicile et une promotion de l'hygiène au programme thérapeutique ambulatoire standard de la malnutrition aiguë sévère sur les taux de guérison dans le nord du Sénégal ».**

***Professeur***,

J'accuse réception de vos réponses aux questions relatives au protocole en référence ci-dessus. À l'analyse, le Comité National d'Ethique pour la Recherche en Santé les trouve globalement satisfaisantes. En conséquence, le comité émet un avis éthique et scientifique favorable pour permettre la mise en œuvre dudit protocole.

Cet avis a une durée d'une année à compter de sa date de signature. Son renouvellement reste assujetti à la présentation d'un rapport d'étape permettant d'être informé sur le niveau de mise en œuvre de l'étude. Un suivi de ladite étude sera programmé en rapport avec votre structure.

Je vous prie de croire, ***Professeur***, à l'assurance de ma considération distinguée et de mes encouragements renouvelés.

COMITE NATIONAL D'ETHIQUE POUR LA RECHERCHE EN SANTE
Pr Anta Tal DIA
La [redacted]

***Pr. Oliver Cumming***
***Chercheur Principal de l'Etude***
***London School Hygiène & Tropical Médicine***

**London School of Hygiene & Tropical Medicine**
Keppel Street, London WC1E 7HT
United Kingdom
Switchboard: +44 (0)20 7636 8636

**www.lshtm.ac.uk**

**Observational / Interventions Research Ethics Committee**

Mr Oliver Cumming
LSHTM

4 June 2019

Dear Mr Oliver Cumming ,

**Study Title:** The TISA Trial: Effect of WASH and OTP for SAM recovery in Senegal

**LSHTM ethics ref:** 17511

Thank you for your application for the above research, which has now been considered by the Interventions Committee.

**Confirmation of ethical opinion**

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

**Conditions of the favourable opinion**

Approval is dependent on local ethical approval having been received, where relevant.

**Approved documents**

The final list of documents reviewed and approved by the Committee is as follows:

| Document Type | File Name | Date | Version |
|---|---|---|---|
| Other | Human Tissue Certficate_Grignard | 29/04/2019 | 1 |
| Other | HTA_certificate_Gallandat | 29/04/2019 | 1 |
| Other | Human Tissue Training Certificate_Cumming | 29/04/2019 | 1 |
| Investigator CV | CV (2pp)_Cumming_UIF | 29/04/2019 | 1 |
| Investigator CV | CV Moustapha SEYE | 29/04/2019 | 1 |
| Investigator CV | CV_Anglais_Dieynaba N'Diaye_2018.09.29 | 29/04/2019 | 1 |
| Other | GCP certifcate_Oliver Cumming | 29/04/2019 | 1 |
| Information Sheet | TISA_Consent_29.04.2019 | 29/04/2019 | 1 |
| Information Sheet | TISA_Participant Information Sheet_29.04.2019 | 29/04/2019 | 1 |
| Protocol / Proposal | TISA_Protocol_29.04.2019 | 29/04/2019 | 1 |
| Protocol / Proposal | TISA_Questionnaires_29.04.2019 | 29/04/2019 | 1 |
| Sponsor Letter | 2019-KEP-267_sponsor confirmation_30042019 | 29/04/2019 | 1 |

**After ethical review**

The Chief Investigator (CI) or delegate is responsible for informing the ethics committee of any subsequent changes to the application. These must be submitted to the Committee for review using an Amendment form. Amendments must not be initiated before receipt of written favourable opinion from the committee.

The CI or delegate is also required to notify the ethics committee of any protocol violations and/or Suspected Unexpected Serious Adverse Reactions (SUSARs) which occur during the project by submitting a Serious Adverse Event form.

An annual report should be submitted to the committee using an Annual Report form on the anniversary of the approval of the study during the lifetime of the study.

At the end of the study, the CI or delegate must notify the committee using an End of Study form.

All aforementioned forms are available on the ethics online applications website and can only be submitted to the committee via the website at: http://leo.lshtm.ac.uk

Additional information is available at: www.lshtm.ac.uk/ethics

Yours sincerely,

**Professor John DH Porter**
**Chair**

ethics@lshtm.ac.uk
http://www.lshtm.ac.uk/ethics/

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**Improving health worldwide**

# Appendix 4

Appendix 4: Participant Information Sheet and Consent Form for TISA trial

**INFORMED CONSENT FORM**

**TISA Trial: A cluster randomized controlled trial for the effect of adding household water treatment and hygiene promotion to standard outpatient therapeutic treatment of severe acute malnutrition in northern Senegal**

Principal Investigators: Oliver Cumming (LSHTM), Dieynaba N'Diaye (ACF), Moustapha Seye (LARTES)

| | **Please initial each box** |
|---|---|
| 1. I confirm that I have read and understand the participant information sheet dated XX.XX.XXXX (Version 1) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered fully. | |
| 2. I understand that my participation is voluntary and I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. | |
| 3. I understand that sections of the medical notes of the child for which I am responsible, and data collected during the study, may be looked at by responsible individuals from the London School of Hygiene & Tropical Medicine, where it is relevant to their participation in this research. I give permission for these individuals to access those records. | |
| 4. I agree to take part in the above study. | |

| Name of Participant *(printed)* | Signature/Thumbprint | Date (dd/mm/yyyy) |
|---|---|---|
| Name of Person taking consent (*printed*) | Signature | Date (dd/mm/yyyy) |

**Only if the participant does not read or speak French (otherwise enter "N/A" in fields):** As a witness, I confirm that the translation of the information about the study from French to Wolof or Pular was accurate.

| Name of Impartial Witness (*printed*) | Signature | Date |
|---|---|---|

**If the participant is unable to sign (otherwise enter "N/A" in fields):** As a witness, I confirm that all the information about the study was given and the participant consented to taking part.

| Name of Impartial Witness (*printed*) | Signature | Date (dd/mm/yyyy) |
|---|---|---|

*1 copy for participant; 1 copy for study team to be retained*

**PARTICIPANT INFORMATION SHEET**

Study title:

**TISA: A cluster randomized controlled trial for the effect of adding household water treatment and hygiene promotion to standard outpatient therapeutic treatment of severe acute malnutrition in northern Senegal**

You are being invited to take part in a research study led by the London School of Hygiene and Tropical Medicine in the United Kingdom, Action Contre le Faim Senegal and LARTES, in collaboration with the Ministry of Health for Senegal. Before you decide whether to participate, it is important for you to understand why the research is being done and what it will involve. Please ask us if there is anything that is not clear or if you would like more information, and please take time to decide whether or not you wish to take part.

The following is to explain the details of the study:

**1. What is the purpose of the study?**

Severe acute malnutrition (SAM) affects many children in northern Senegal and neighbouring countries. Malnutrition is often associated with enteric infections and diarrhoea, and which can be prevented by the use of safe drinking water and adequate hygiene practices such as handwashing. The purpose of the study is to find out whether giving caregivers a product for household water treatment (Aquatabs) along with a safe water storage container, along with information and training about how to treat the water, and practice key hygiene behaviours, can help children's recovery from SAM. If

**2. Why have I been chosen?**

You are being asked to participate in this study because your child has severe acute malnutrition (SAM) and you sought care at one of the health posts (UREN) included in the study. All health posts (UREN) from the departments of Linguère and Podor are included in the study and we are inviting all caregivers of children diagnosed with SAM to participate.

**3. Do I have to take part?**

It is up to you to decide whether to join the study. We will describe the study and go through this information sheet and, if you agree to take part, we will then ask you to sign a consent form. You are free to withdraw from the study at any time, without giving a reason. If you decide to withdraw, with your permission, we will record your reason for not participating. Your decision to join the study or not will not affect the health services that are available to you or your child.

**4. What will happen to me if I take part?**

Health posts in the study area – the departments of Linguère and Podor – have been randomly assigned to one of two groups. Patients seeking care at UREN in Group 1 will receive standard treatment for severe acute malnutrition (SAM) and a "kit" that contains a supply of water treatment product (Aquatabs) and with a safe water storage container, along with explanations on how to use these products and maintain a hygienic environment in their home. Patients seeking care at UREN in Group 2 will receive standard treatment for severe acute malnutrition (SAM), and at the end of the study (8 weeks after enrolment) will be provided with the same kit.

Independent of whether you are in Group 1 or Group 2, we will ask you to take part in five activities:

a. Today, if you agree to participate in the study, we will ask you questions regarding your household, and regarding the living conditions in your household and village or neighbourhood, including questions on electricity, water and sanitation. This will take no more than 15 minutes of your time and we will conduct the survey here at the health post.

b. The health post staff will ask you to come back every week with your child until he/she recovers from severe acute malnutrition (SAM). This is the standard national protocol for SAM treatment. At each visit, health post staff will evaluate your child's health condition and record information.

c. Study personnel will visit your household twice in the coming weeks for a short when we will ask you a series of questions and you may be asked to provide a sample of your drinking water.

d. When your child ends SAM treatment, another short survey will be conducted at the UREN and the nurse will explain the procedure and ask permission to take a stool sample (rectal swab) from your child. This will take no more than 30 minutes of your time.

f. 8 weeks after enrolment we will ask you to return to the UREN so that we can take a stool sample from your child by rectal swab. This procedure will be explained to you by a nurse before it is performed and you can decline for your child to participate at any time.

**5. What are the possible disadvantages and risks of taking part?**

Collecting the stool sample will cause no more than minimal discomfort to your child and does not pose a risk to your child. The sample will be taken by trained medical staff at the local health post which you attend for SAM treatment and all procedures will be overseen by trained medical staff.

**6. What are the possible benefits of taking part?**

Participation in the study will not help you or your child directly but the information we collect may lead to improvements in how severe acute malnutrition (SAM) is managed in Senegal and support more effective strategies which lead to better outcomes. If the UREN you attend for SAM treatment is in Group 1, you will receive a kit containing household water treatment products, a safe water storage container, and training on how to maintain a hygienic environment in your home. If the UREN you attend is in Group 2, you will receive the same kit 8 weeks after enrolment when you return to the UREN.

**7. Will my taking part in the study be kept confidential?**

Yes. All information collected about you and your child during the course of the research will be kept strictly confidential, and all data will be anonymised before analysis and publication.

**8. What will happen if I don't want to carry on with the study?**

You can decide to stop participating in the study at any time, even after your child has provided samples. If you withdraw from the study, you can decide whether you want us to destroy the questionnaire and all samples taken from your children or the household, or whether you allow us to use these previously-collected data.

**9. Who will pay the costs that I may incur through participating in this study?**

The only costs you will incur as a result of participating in the study are your travel costs for attending the UREN 8 weeks after enrolment for the stool sample to be collected. Your travel costs for this will be reimbursed.

**10. What if something goes wrong?**

If you have a concern about any aspect of this study, you should ask to speak to the local study supervisor, Dr Arona Diene, who will try to answer your questions. If you wish to complain formally, or have any concerns about any aspect of the way you have been treated, you should immediately inform the Principal Investigators:

Oliver Cumming: oliver.cumming@lshtm.ac.uk, +44 20 7636 8636
Dieynaba N'Diaye: dndiaye@actioncontrelafaim.org, +33 1 70 84 72 54
Moustapha Seye: cmoustaphaseye@gmail.com +221 77 438 87 02

The London School of Hygiene & Tropical Medicine holds insurance policies which apply to this study. If you experience harm or injury as a result of taking part in this study, you may be eligible to claim compensation.

**12. What will happen to the samples and the results of the research study?**

Stool samples (rectal swabs) will be collected at the health post, then prepared for shipping at a local laboratory, and sent to the United Kingdom for analysis, without your child's name on it. Samples may be stored in a freezer until analysis and will be tested for the presence of microbes (pathogens) that can cause gastrointestinal infections.

If a water sample is collected from your household, that sample will be analysed at a local laboratory for the presence of bacteria (coliforms) used as indicators of faecal contamination in the water. All the data from the questionnaires and the samples will be analysed by researchers working with the London School of Hygiene and Tropical Medicine. Overall results of the study will be summarized anonymously and will only be presented in aggregated form, without any identifying information. It is expected that study results will be published and shared after completion of the study with partner organizations, including Action Contre la Faim and the Ministry of Health and Social Action for Senegal.

**11. Contact Details**

**Local emergency contact number: Study Coordinator:**

Dr Arona Diene, Action Contre la Faim, Louga, Senegal. Tel. +221 77 294 9556.

**If you have any questions about your rights as a research participant, please contact:**

Dr Samba Cor Sarr, National Health Research Ethics Committee (CNERS), 2e étage Siège du Ministère de la Santé et de l'Action Sociale, Rue 1 Aimé-Césaire, BP 4024, Dakar, Fann. Tel. +221 33 869 4313.

**You will be given a copy of the information sheet and a signed consent form to keep.**

**Thank you for considering taking the time to read this sheet.**

# Appendix 5

Appendix 5: TaqMan Array Card gene targets and sequences for TISA trial

**Appendix 5: TaqMan Array Card molecular gene targets and sequences**

| Category | Pathogen | Target | reference | Primers & probes sequences (labelled with FAM (6-carboxyfluorescein) at 5' and MGB at 3') |
|---|---|---|---|---|
| **Virus** | Adenovirus F (40/41) | fiber gene | (1) | F: AACTTTCTCTCTTAATAGACGCC; R: AGGGGGCTAGAAAACAAAA<br>Probe: CTGACACGGGCACTCT |
| | Astrovirus | Capsid | (2) | F: CAGTTGCTTGCTGCGTTCA; R: CTTGCTAGCCATCACACTTCT<br>Probe: CACAGAAGAGCAACTCCATCGC |
| | Norovirus GI | ORF1-2 | (1) | F: CGYTGGATGCGNTTYCATGA; R: CTTAGACGCCATCATCATTYAC<br>Probe: TGGACAGGAGATCGC |
| | Norovirus GII | ORF1-2 | (2,3) | F: CARGARBCNATGTTYAGRTGGATGAG; R: TCGACGCCATCTTCATTCACA<br>Probe: TGGGAGGGCGATCGCAATCT |
| | Rotavirus | NSP3 | (2,4) | F: ACCATCTWCACRTRACCCTCTATGAG; R: GGTCACATAACGCCCCTATAGC<br>Probe: AGTTAAAAGCTAACACTGTCAAA |
| | Sapovirus | RdRp | (2) | Fw1: GAYCASGCTCTCGCYACCTAC; Fw2: TTGGCCCTCGCCACCTAC;<br>R: CCCTCCATYTCAAACACTA<br>Probe: CCRCCTATRAACCA |
| **Bacteria** | Aeromonas | aerolysin | (2) | F: TYCGYTACCAGTGGGACAAG; R: CCRGCAAACTGGCTCTCG<br>Probe: CAGTTCCAGTCCCACCACTT |
| | Campylobacter jejuni/coli | cadF | (2,5) | F: CTGCTAAACCATAGAAATAAAATTTCTCAC; R: CTTTGAAGGTAATTTAGATATGGATAATCG<br>Probe: CATTTTGACGATTTTTGGCTTGA |
| | C. difficile | tcdB | (2) | F: GGTATTACCTAATGCTCCAAATAG; R: TTTGTGCCATCATTTTCTAAGC<br>Probe: CCTGGTGTCCATCCTGTTTC |
| | EAEC | aaiC | (2,6) | F: ATTGTCCTCAGGCATTTCAC; R: ACGACACCCCTGATAAACAA<br>Probe: TAGTGCATACTCATCATTTAAG |
| | EAEC | aatA | (2,6) | F: CTGGCGAAAGACTGTATCAT; R: TTTTGCTTCATAAGCCGATAGA<br>Probe: TGGTTCTCATCTATTACAGACAGC |
| | EAEC | aggR | (1,7) | F: GCAATCAGATTAARCAGCGATACA; R: TTCGGACAACTRCAAGCATC<br>Probe: AAGACGCCTAAAGGATGCCC |
| | STEC | stx1 | (2) | F: ACTTCTCGACTGCAAAGACGTATG; R: ACAAATTATCCCCTGWGCCACTATC<br>Probe: CTCTGCAATAGGTACTCCA |
| | STEC | stx2 | (2,8) | F: CCACATCGGTGTCTGTTATTAACC; R: GGTCAAAACGCGCCTGATAG<br>Probe: TTGCTGTGGATATACGAGG |
| | EPEC | Eae | (2) | F: CATTGATCAGGATTTTTCTGGTGATA; R: CTCATGCGGAAATAGCCGTTA<br>Probe: ATACTGGCGAGACTATTTCAA |

| | | | | |
|---|---|---|---|---|
| | EPEC | bfpA | (1,2) | F: TGGTGCTTGCGCTTGCT; R: CGTTGCGCTCATTACTTCTG<br>Probe: CAGTCTGCGTCTGATTCCAA |
| | ETEC | LT | (2,8) | F: TTCCCACCGGATCACCAA; R: CAACCTTGTGGTGCATGATGA<br>Probe: CTTGGAGAGAAGAACCCT |
| | ETEC | STh | (2) | F: GCTAAACCAGYAGRGTCTTCAAAA; R: CCCGGTACARGCAGGATTACAACA<br>Probe: TGGTCCTGAAAGCATGAA |
| | ETEC | STp | (2) | F: TGAATCACTTGACTCTTCAAAA; R: GGCAGGATTACAACAAAGTT<br>Probe: TGAACAACACATTTTACTGCT |
| | E. coli O157 | rfbE | (1,9( | F: TTTCACACTTATTGGATGGTCTCAA; R: CGATGAGTTTATCTGCAAGGTGAT<br>Probe: CTCTCTTTCCTCTGCGGTCCT |
| | Helicobacter pylori | ureC | (1) | F: GACACCAGAAAAAGCGGCTA; R: AGCGCATGTCTTCGGTTAAA<br>Probe: TCACTAAAGCGTTTTCTACC |
| | Plesiomonas shigelloides | gyrB | (1) | F: CCGCCGTGAAGGCAAAG; R: GCTACCGGCTCACCCAGAT<br>Probe: CACACCCAAGAATAC |
| | Salmonella enterica | ttr | (1,10) | F: CTCACCAGGAGATTACAACATGG; R: AGCTCAGACCAAAAGTGACCATC<br>Probe: CACCGACGGCGAGACCGACTTT |
| | Salmonella enterica Typhi | STY0201 | (1,11) | F: CGCGAAGTCAGAGTCGACATAG; R: AAGACCTCAACGCCGATCAC<br>Probe: CAGCCTGCTCCAGAACA |
| | Shigella/EIEC | ipaH | (1,12) | F: CCTTTTCCGCGTTCCTTGA; R: CGGAATCCGGAGGTATTGC<br>Probe: CGCCTTTCCGATACCGTCTCTGCA |
| | Shigella flexneri | Putative periplasmic Protein* | (13) | F: TGGGTGCATCCTGACCTGT; R: GACAAACAATAACGAGCTACCGAT<br>Probe: ACCACGGAATAATCCCGCAG |
| | Shigella flexneri | O-antigen** | (13) | F: CTCCTATCCGTGATTATAGTGCA; R: GCACACACAACTCACTGTATTT<br>Probe: TCCTTCTCACGATTAAAATC |
| | Shigella flexneri | Type 3 restriction Enzyme** | (13) | F: CTTTCAACGCACGAATATCAAC; R: GAACCTGATCCAGACGGAGA<br>Probe: TTCTTCAGAACCGGGTTTTG |
| | Shigella sonnei | Putative methylase | (13) | F: TGCCGCTAAAATCCTTCTGT; R: GCGTACGACGAAAGGAAAAA<br>Probe: GAAGTTATTGATTCCGCCC |
| | Vibrio cholerae | hlyA | (1) | F: ATCGTCAGTTTGGAGCCAGT; R: TCGATGCGTTAAACACGAAG<br>Probe: ACCGATGCGATTGCCCAA |
| | Vibrio cholerae | ctxA | (14) | F: GCATAGAGCTTGGAGGGAAGAG; R: CATCGATGATCTTGGAGCATTC<br>Probe: CATCATGCACCGCCG |
| | Yersinia enterocolitica | lytA | (1,15) | F: TGATTCACCAGCAGCAATAC; R: GGCATCATGAAAGGCGG<br>Probe: TGTCGGTTTCTCCTTCCAGG |

| | | | | |
|---|---|---|---|---|
| **Protozoa** | Cryptosporidium spp. | 18S rRNA | (1) | F: GGGTTGTATTTATTAGATAAAGAACCA; R: AGGCCAATACCCTACCGTCT<br>Probe: TGACATATCATTCAAGTTTCTGAC |
| | Cyclospora cayetanensis | 18S | (1) | F: AAAAGCTCGTAGTTGGATTTCTG; R: AACACCAACGCACGCAGC<br>P: AAGGCCGGATGACCACGA |
| | Giardia spp. | 18S rRNA | (2,16) | F: GACGGCTCAGGACAACGGTT; R: TTGCCAGCGGTGTCCG<br>Probe: CCCGCGGCGGTCCCTGCTAG |
| | E. histolytica | 18S rRNA | (2,16) | F: ATTGTCGTGGCATCCTAACTCA; R: GCGGACGGCTCATTATAACA,<br>Probe: TCATTGAATGAATTGGCCATTT |
| **Helminth** | Ascaris lumbricoides | ITS1 | (1) | F: GCCACATAGTAAATTGCACACAAAT; R: GCCTTTCTAACAAGCCCAACAT<br>Probe: TTGGCGGACAATTGCATGCGAT |
| | Trichuris trichiura | 18S rRNA | (2) | F: TTGAAACGACTTGCTCATCAACTT; R: CTGATTCTCCGTTAACCGTTGTC<br>Probe: CGATGGTACGCTACGTGCTTACCATGG |
| | Ancylostoma duodenale | ITS2 | (1,17) | F: GAATGACAGCAAACTCGTTGTTG; R: ATACTAGCCACTGCCGAAACGT<br>Probe: ATCGTTTACCGACTTTAG |
| | Necator americanus | ITS2 | (1,17) | F: CTGTTTGTCGAACGGTACTTGC; R: ATAACAGCGTGCACATGTTGC<br>Probe: CTGTACTACGCATTGTATAC |
| | Strongyloides stercoralis | dispersed repetitive sequence | (1,18) | F: TCCAGAAAAGTCTTCACTCTCCAG; R: TGCGTTAGAATTTAGATATTATTGTTGCT<br>Probe: TCAGCTCCAGTTGAACAACAGCCTCCAA |
| | Schistosoma spp. | ITS | (1,19) | F: GGTCTAGATGACTTGATYGAGATGCT; R: TCCCGAGCGYGTATAATGTCATTA<br>P: TGGGTTGTGCTCGAGTCGTGGC |
| **Other/ virus** | SARS-CoV-2# | N1 | (20) | F: GACCCCAAAATCAGCGAAAT; R: TCTGGTTACTGCCAGTTGAATCTG<br>Probe: ACCCCGCATTACGTTTGGTGGACC |
| **Other/ virus** | SARS-CoV-2# | E-Sarbeco | (21) | F: ACAGGTACGTTAATAGTTAATAGCGT; R: ATATTGCAGCAGTACGCACACA<br>Probe: ACACTAGCCATCCTTACTGCGCTTCG |
| **Control/ RNA virus** | MS2 | MS2g1 | (2,22) | F: TGGCACTACCCCTCTCCGTATTCAC; R: GTACGGGCGACCCCACGATGAC<br>Probe: CACATCGATAGATCAAGGTGCCTACAAGC |
| **Control/ DNA virus** | PhHV | gB | (2) | F: GGGCGAATCACAGATTGAATC; R: GCGGTTCCAAACGTACCAA<br>Probe: TATGTGTCCGCCACCATCT |
| **Control/ 16S rRNA** | 16S | 16S | (1,23) | F: TGCAAGTCGAACGAAGCACTTTA; R: GCAGGTTACCCACGCGTTAC<br>Probe: CGCCACTCAGTCACAAA |
| **Control/ 18S rRNA** | 18S† | 18S | N/A | Manufacturer's control |

*This assay detects most *S. flexneri* serotypes except for serotype 6.

**The combination of these two assays identifies *S. flexneri* serotype 6 when both are positive (Cq≤35)

# Included on the TAC but not reported in this manuscript as not a enteric pathogen primarily transmitted via faecal-oral route.

†ThermoFisher manufacturer control

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