LINKAGE BETWEEN GUT HEALTH AND NUTRITIONAL STATUS AMONG CHILDREN AGED 12-15 MONTHS WITH COMPROMISED WATER, SANITATION AND HYGIENE ACCESS IN SIAYA COUNTY, KENYA

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A Thesis Submitted to the School of Public Health, Biomedical Sciences and Technology in Partial Fulfilment of the Requirements for the Award of Master of Science in Medical Dietetics Degree of Masinde Muliro University of Science and Technology

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DECLARATION

This thesis document is my original work and has not been presented in any other institution for examination or any other purposes

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SUPERVISORS' APPROVAL

The undersigned certify that they have read and hereby recommend for acceptance of Masinde Muliro University of Science and Technology a thesis entitled "***Linkage between gut health and nutritional status among children aged 12-15 months with compromised water, sanitation and hygiene access in Siaya County, Kenya"***.

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DEDICATION

This thesis is dedicated to my loving wife Mercy, whose unwavering support and encouragement have been my pillar of strength throughout this journey. Your patience, understanding, and belief in me have been invaluable. To our future children, may this work inspire you to pursue your dreams with passion and determination. To my parents, whose sacrifices, love, and guidance have shaped the person I am today. Your unwavering faith in me has been a constant source of motivation. Finally, to all the mothers and their children in resource limited settings to whom access to water, sanitation and hygiene facilities is a daily struggle, I am grateful.

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ABSTRACT

The Kenyan government has implemented programs to improve child nutrition, yet malnutrition persists in Siaya County, where stunting affects 19% of children, higher than in other regions. Poor access to water, sanitation, and hygiene (WASH) in these areas exposes children to enteric pathogens, leading to environmental enteric dysfunction (EED). Characterized by gut inflammation, impaired absorption, and increased permeability, EED significantly impacts nutritional status, contributing to persistent undernutrition, particularly in rural areas. Despite the critical role of gut health, it is often overlooked in managing childhood malnutrition. This study investigated the relationship between gut health and nutritional status among children aged 12-15 months compromised access WASH in Siaya County, Kenya. The study assessed the WASH situation, gut health and nutrition status among the children. A cross-sectional analytical study was conducted among 100 children at Siaya County Referral Hospital, selected purposively for its referral role. Children were recruited via simple random sampling during child clinics. WASH data was collected using a semi-structured questionnaire, while nutritional status was assessed through anthropometric measurements. Gut health was evaluated using the lactulose and rhamnose sugars ratio and the 13-carbon sucrose breath test to diagnose EED. Descriptive analysis employed percentages, means and standard deviations while inferential statistics used chi-square tests, Pearson's correlation, independent t-tests, ANOVA, and simple linear regression analysis were used for inferential statistics. Most respondents were married (80%), with 19% single. Education levels showed concerning trends with 40% having only primary education, 42% secondary, and with smaller proportion (18%) attaining tertiary level. Overall, 76% of the households had improved sources of water indicating some access to safe and protected water sources. However, 22% utilized surface water, increasing the risk to enteric infections. Further, sanitation was the major concern with 90% having unimproved toilet facilities. Additionally, 62% had no separate room as kitchen, and 69.2% lacked a handwashing facilities, with only 30% using soap. The prevalence of EED was high at 53%, with a mean lactulose and rhamnose ratio of 3.03 ± 4.32, indicating significant gut impairment. The 13-carbon sucrose breath test further revealed intestinal damage, with a mean change of -0.45 in the 13-carbon recovered between baseline and 90 minutes, suggesting impaired intestinal mucosa. High rates of malnutrition were observed, with 45% of children affected by wasting, 20% by stunting, and 14% by underweight. Significant negative correlations were found between soap use ($p=0.02$), handwashing stations (0.029), and gut health status, implying that availability of these sanitation facilities in households reduced risk of gut impairment. Regression analysis indicated significant link between water sources for households and impaired gut health ($p=0.039$). However, gut health was not significantly related to nutritional status indicators ($p>0.05$), indicating a brush border effect on the gut. In conclusion, poor WASH contributes to impaired gut health, affecting children's nutritional status. Nutrition interventions by stakeholders and policy makers should consider gut health in developing guidelines for management of undernutrition.

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ABBREVIATIONS AND ACRONYMS

^{13}C-SBT	**:**	13 Carbon Sugar Breath test
CIDP	**:**	County Integrated Development Program
COVID-19	**:**	Corona Virus Disease 2019
CPDR	**:**	Cumulative Percent Dose Rate
DDS	**:**	Dietary Diversity scores
EED	**:**	Environmental Enteric Dysfunction
HAZ	**:**	Height –for –Age Z-Scores
IAEA	**:**	International Atomic Energy Agency
KDHS	**:**	Kenya Demographic Health Survey
KNBS	**:**	Kenya National Bureau of Statistics
LRR	**:**	Lactulose: Rhamnose Ratio
MCH	**:**	Maternal and Child Health
MOH	**:**	Ministry of Health
SCTRH	**:**	Siaya County Teaching and Referral Hospital
SDG	**:**	Sustainable Development Goals
SSA	**:**	Sub- Saharan Africa
UK	**:**	United Kingdom
UNICEF	**:**	United Nations International Children's Fund
USA	**:**	United States of America
USAID	**:**	United States Agency for International Development
WASH	**:**	Water, Sanitation and Hygiene
WAZ	**:**	Weight –for –Age Z-Scores
WHO	**:**	World Health Organization
WHZ	**:**	Weight –for- Height Z-Scores

DEFINITION OF OPERATIONAL TERMS

Gut Health :	Multiple positive aspects of the gastrointestinal Tract (GIT), such as the effective digestion, absorption and, absence of illness. Determined by presence/absence of Environmental Enteric Dysfunctions (EED)
Stunting :	Under-nutrition for a long time, which retards the growth of a child by height. The child is shorter for its age
Underweight :	Composite indicator of long term and acute short- term malnutrition. The body weight may be lost due to malnutrition for a long time
Wasting :	Wasting: Acute malnutrition indicated by low weight for height, a sign for rapid weight loss or inadequate weight gain.
Nutritional status :	Health of person as influenced by the quantity and quality of foods eaten and the ability of body to utilize these foods to meet its needs as per age, activity pattern and physiological conditions of the person
Environmental Enteric Dysfunction :	A subclinical condition resulting from the inflammation and disruption of the absorptive ability and permeability of the gut
Under-five children :	Young children of the age less than 5 years
Undernutrition :	Refers inadequate nutrient intake resulting from limited or lack of food
Child growth :	Increase in height or weight of children with age and as indicated by standard normal deviates (Z-scores) using WHO Child growth Standards
Compromised WASH:	Refers to inadequate or insufficient access to clean water, proper sanitation facilities, and basic hygiene practices.

CHAPTER ONE: INTRODUCTION

This chapter presents the study's background, problem statement, objectives, and research questions, along with the hypotheses. It also addresses the study's justification, and significance, and discusses the conceptual framework.

1.1 Background of the study

Safe drinking water, basic sanitation, and good hygiene practices are crucial for children's survival (Chavura *et al*., 2022; UNICEF, 2023). Globally, 2.2 billion people lack access to safe drinking water, nearly half lack adequate sanitation, two billion lack handwashing facilities with soap, and 419 million practice open defecation, with rural and low-income populations being particularly vulnerable (UNICEF & WHO, 2023; USAID, 2023). Chronic exposure of children to enteric pathogens and parasites due to poor Water, Sanitation, and Hygiene (WASH) conditions is the leading cause of Environmental Enteric Dysfunction (EED). EED is an acquired small intestinal disorder characterized by gut inflammation, reduced absorptive surface area, and disruption of gut barrier function (Regassa *et al*., 2023; Tickel *et al*., 2022). This condition severely impacts children's gut health, impairing nutritional status by reducing nutrient absorption and increasing nutrient losses (McQuade *et al*., 2020; Owino *et al*., 2016; Wolf *et al*., 2023).

The global prevalence of EED among children ranges from 50% to 90%, with the highest rates reported in Asia and Sub-Saharan Africa (Ali *et al*., 2016; Chen *et al*., 2021). In Peru, EED prevalence is 53%, while in Malawi, it reaches 83% among children under five years (Ordiz *et al*., 2016; Faubion *et al*., 2016). Conversely, lower prevalence's have been observed in Tanzania and the United Stated of America (USA), at 9% and 5%

respectively, among children aged one to two years (Modern *et al*., 2022; Faubion *et al.*, 2016).

The high burden of EED in many developing countries is often associated with reduced effectiveness of nutritional interventions and oral vaccines, stemming from impaired intestinal absorptive capacity (Korpe & Petri, 2012; Marie et al., 2018). EED related gut damage, marked by villous blunting and inflammatory cell infiltration, results in mild nutrient malabsorption and increased intestinal permeability (Tickel *et al*., 2022). This condition leads to, chronic systemic immune activation and diverts energy from growth, further impairing children's development (Jamil *et al.*, 2021; Owino *et al*., 2021; Tickell *et al*., 2022)

Globally, there has been a slow improvement in the nutrition status of children, as of 2022, stunting was 149 million (22.3%), a decline of 30 million from 2012, with the majority in Asia (52%) and Africa (43%). Wasting was at 45 million (6.8%), which is highest in low and middle-income countries that represent 94% of the burden, with Asia contributing 70% and Africa 27% of the wasting (FAO., 2023; UNICEF., 2023). The prevalence of underweight is at 14.7% with little change over two decades, 16.6% in the year 2000 (FAO., 2023). In East Africa, stunting is 35.3% and wasting is 5.6% (Quamme & Iversen, 2022).

The 2022 Kenya Demographic Health Survey (KDHS) indicates that 59% of the population has adequate access to safe drinking water. However, only 29% have access to basic sanitation, reflecting a 5% decline since 2000. Approximately 9.9 million individuals rely on contaminated water sources, and five million practice open defecation. Furthermore, only 25% of the population has access to handwashing facilities (KDHS, 2022). In Siaya County, only 49% of the population has access to safe drinking water, and just 35% have basic sanitation. Additionally, 44.2% lack pit latrines, with 19% resorting to open defecation. About 50% of illnesses treated in Kenyan hospitals are linked to water, sanitation, and hygiene issues, particularly gut health problems like diarrhea, enteric infections, and intestinal parasites, which hinder effective nutrient absorption. This situation is likely exacerbated by the rapidly growing population, which strains existing sanitation facilities and safe drinking water sources (KDHS, 2022; Osiemo *et al*., 2019).

In Kenya, stunting among children under five stands at 18%, down from 26% in 2014, while wasting has increased to 5% from 4%, and underweight has dropped to 10% from 11%. Although malnutrition rates have changed, the growing population since 2014 may mask genuine progress in addressing the issue. Siaya County has slightly higher stunting rates at 19% but lower wasting (2%) and underweight (7%) rates compared to national statistics (KDHS, 2022; KDHS, 2014). Despite the WASH and nutritional interventions by the Kenya Ministry of Health (MOH) to reduce undernutrition among under-fives, high stunting, wasting, and underweight rates persist. Researchers have linked inadequate WASH practices and impaired gut health to poor nutritional status, however the current guidelines on management of malnutrition does not incorporate the gut health status (George *et al*., 2018; Kwami *et al.*, 2019; Lazar *et al*., 2024). Therefore, the study investigated the link between gut health and nutrition status among children with compromised access to WASH. The findings may be key in formulating policies to manage child malnutrition effectively and implement programs to improve child nutrition status.

1.2 Problem statement.

The high prevalence of poor WASH conditions in Kenya, particularly in rural areas, significantly impacts children's gut health due to their chronic exposure to enteric pathogens and parasites (Humphrey *et al*., 2018; KDHS, 2022; UNICEF, 2023). This exposure is a major contributor to EED, a condition that impairs the gut's lining and nutrient absorptive capacity, resulting in poor nutritional status among affected children (Tickell *et al.,* 2022; Modern *et al*., 2022; Vilcins *et al*., 2018).

Despite interventions such as therapeutic diets and supplementation, the persistence of stunting, wasting, and underweight in Kenyan children, along with prolonged hospitalizations and, in some cases, deaths indicate that this measures alone may be insufficient. Emerging research suggests that impaired gut health may be a critical factor in children's limited response to nutritional interventions, particularly within the first two years of life (Lassi *et al*., 2013; McQuade *et al.*, 2020).

Although gut health status is essential for the effectiveness of child nutrition interventions, it is currently excluded from Kenya's management guidelines for malnutrition *(*Arnold *et al.*, 2013; Lauer *et al*., 2020). This gap is particularly concerning in Siaya County, where compromised WASH conditions are prevalent and likely exacerbate gut health issues associated with poor nutrition indicators. However, there is limited research on the relationship between children's gut health and their nutritional status in this region. Understanding this linkage is crucial for developing targeted interventions and evidence-based policies to manage malnutrition effectively in the county and country.

1.3 Objectives of the study

1.3.1 Broad objective

The aim of this study was to determine linkage between gut health, and nutrition status among children 12-15 months with compromised water, sanitation and hygiene Siaya County, Kenya.

1.3.2 Specific Objectives

i. To assess the water, sanitation and hygiene situation among children aged 12-15 months, Siaya County, Kenya

ii. To determine the gut health of children 12-15 months in Siaya County,Kenya using the 13-carbon sucrose breath test (13-CSBT)

iii. To evaluate the nutrition status of children aged 12-15 months Siaya County, Kenya.

iv. To investigate the association between water, sanitation and hygiene, gut health and nutrition status among children aged 12-15 months in Siaya County, Kenya.

1.3.3 Research questions

i. What is the water, sanitation and hygiene situation among children aged 12-15 months in Siaya County, Kenya?

ii. What is the gut health of children aged 12-15 months in Siaya County, Kenya?

iii. What is the nutrition status of children aged 12-15 months in Siaya County, Kenya?

iv. What is the relationship between water, sanitation and hygiene, gut health and nutrition status among children aged 12-15 months in Siaya County, Kenya?

1.3.4 Hypothesis

$H0_1$. There is no significant relationship between water, sanitation and hygiene and gut health among children aged 12-15 months in Siaya County, Kenya.

$H0_2$.There is no significant relationship between gut health and nutrition status among children aged 12-15 months in Siaya County, Kenya.

1.4 Justification of the Study

Understanding the interplay between WASH, gut health and nutrition status is critical for improving child health outcomes in Siaya County, Kenya. In Siaya County, to the researcher's best knowledge, there has yet to be a study that has focused on gut health and the nutrition status of children with inadequate access to WASH. Some studies have only explored the link between WASH and children's nutrition status, overlooking the role of gut health. Additionally, some of these studies have methodological limitations, relying on self-reported data collected through semi-structured questionnaires. It may introduce response bias and inaccuracies as individuals overstate or understate their WASH status. Further, such studies were done without using novel diagnostic techniques to assess gut health before linking it to nutrition status, limiting the findings' generalizability (Osiemo *et al*., 2019; Silva *et al*., 2020).

According to the KDHS, (2022), Siaya County has poor access to basic sanitation, safe drinking water, and hygiene practices. Furthermore, the county reports poor nutrition indicators, with little to no research on gut health's role in children's nutrition status. Despite county interventions to improve children nutrition status, health indicators have observed minimal progress (KDHS, 2022). The current study sought to address these gaps in empirical evidence on the linkage between gut health and nutrition status and contribute new knowledge that can be used by researchers, policymakers, stakeholders, and development partners in Siaya County to design and implement effective nutrition interventions among children.

1.5 Significance of the study

The findings on the link between gut health and nutrition status among children with compromised WASH could prompt both county and national governments to invest in non- invasive diagnostic tools for identifying gut health problems such as EED and to develop strategies for its prevention, treatment, and management. This would support the achievement of Sustainable Development Goal (SDG) 3 on good health and SDG 6 on access to quality WASH (UNICEF & WHO, 2023).

Additionally, the study may inform policy improvements for the nutrition management of children under five and enhance WASH conditions through government and private sector initiatives. Reducing the frequency of WASH-related diseases could lower treatment costs and contribute to achieving SDG two, which aims to end malnutrition by 2030 (UNICEF & WHO, 2023). Moreover, this research could enrich the existing literature on WASH, EED, and nutritional status among young children.

1.6 Scope of the study

The study was conducted among children 12-15 months attending Maternal and Child Health (MCH) clinic at Siaya County Referral Hospital (SCRH) in Siaya County, Kenya. The respondents were mothers with children 12-15 months who had accepted and signed an informed consent form. The study endeavored to comprehensively asses WASH practices, gut health and nutrition status among the children in Siaya County. The assessment involved exploring the WASH practices among children including water source for drinking, type of toilet, open defecation and hand washing with clean water and soap. Gut health was determined by Lactulose and Rhamnose Ratio (LRR) and the ^{13}Carbon Sucrose Breath Test (^{13}CSBT). Key anthropometric indicators of height-for-age, weight-for-height, and weight-for-age were assessed, which allowed for the identification of cases of stunted growth, wasting, and underweight conditions among children 12-15 months in Siaya County.

1.7 Limitations of the study

The study was conducted during the COVID-19 pandemic. It negatively affected the 13CSBT study protocol; total time turnaround time was reduced from 240 minutes to 90 minutes. It was amended to reduce the time spent in the hospital and minimize interaction and infection risks. This made comparability with other studies difficult. Another limitation was the dietary habits of the study area, where maize, sugar cane, sorghum, and millet (C4 plants) are predominant food sources. C4 plants metabolize almost all the 13C from the CO2 they absorb, retaining higher levels of the heavy isotope 13C in their tissues. This characteristic influenced the study results, with some baseline (T0) sample results showing higher 13C enrichment than subsequent samples (T2), leading to negative values in some cases. To mitigate this, research assistants probed for the consumption of C4 plant foods in the last 24 hours and implemented subsequent fasting of the children to reduce their effects on the outcomes as noted by Jaika *et al.*, (2024).

1.8 Conceptual framework

The conceptual framework illustrates the intricate relationship between WASH, gut health, and the nutrition status of children. It highlights that inadequate access to clean water, sanitation, and hygiene exposes children to enteric pathogens and parasites. This conditions impair gut health, indicated by presence or absence of EED, negatively impacting child nutrition status. Intervening factors such as child age, gender, child morbidity, parental education level, marital status, parental income and occupation influence have direct or indirect contribution to a child's exposure to EED and consequently poor nutrition status. Dietary intake, diversity, food security, complementary feeding practices also impact the overall nutrition status of a child. All these factors further worsen the effects of compromised WASH on children's gut health and nutrition outcomes (Figure 1.1).

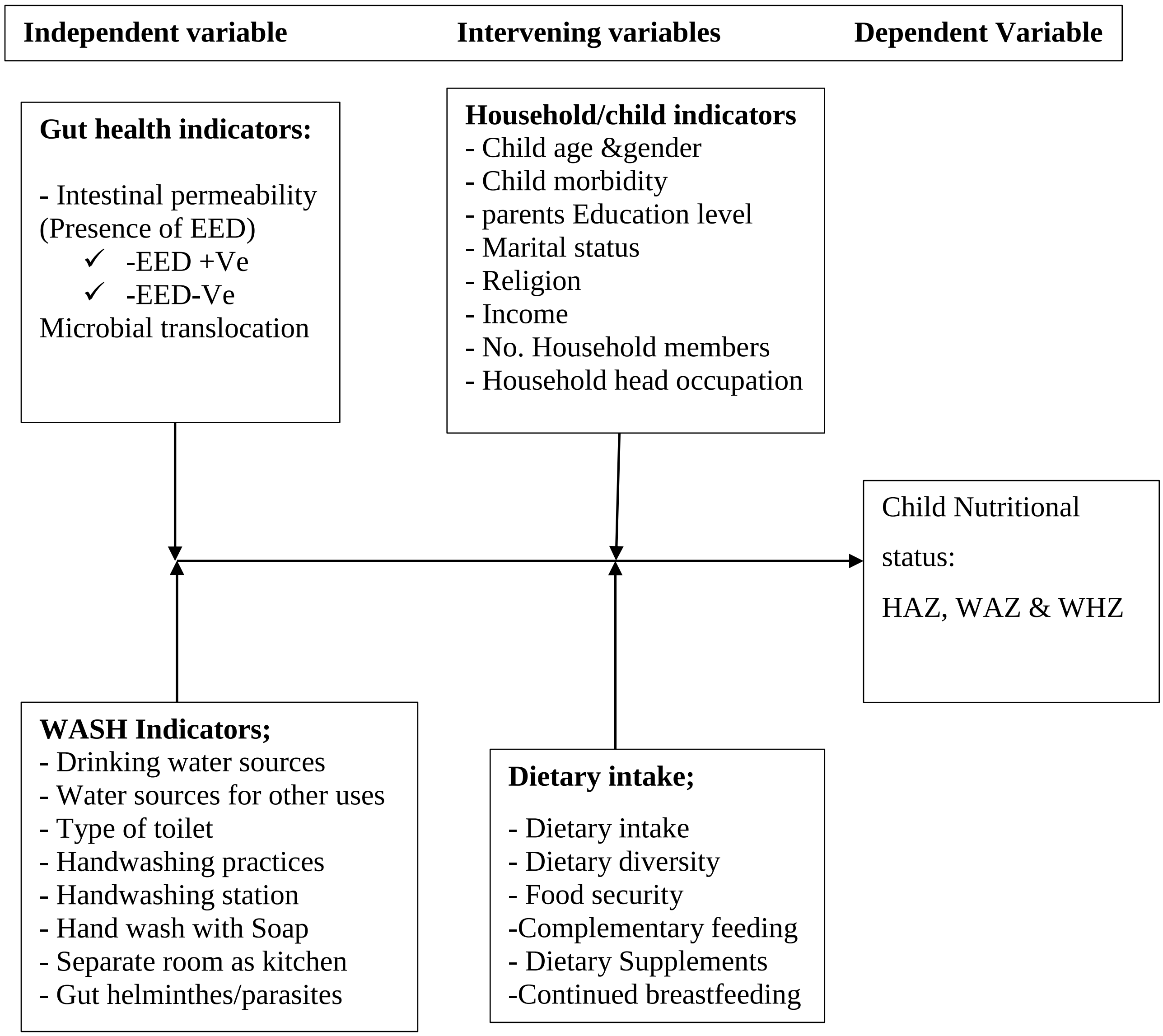

Figure 1.1: **Modified Conceptual framework**

Source: *Model of the mechanisms from poor WASH to EED and child undernutrition (Humphrey et al., 2009)*

CHAPTER TWO: LITERATURE REVIEW

2.0 Introduction

This section presents discussions on WASH, gut health, and nutrition status of under-five children. The review of the literature is presented under the following sub-sections: Water, sanitation hygiene and gut health, and nutrition status of under-fives and analytical techniques for EED.

2.1 Water, sanitation and hygiene in resource limited settings

WASH stand as fundamental pillars to public health and sustainable development Worldwide. Their impact resonates deeply, touching upon the various aspects of the global communities lives, particularly in the developing world; WASH issues continue to present significant challenges in fulfilling the highly prioritized SDG 6 that emphasizes the need to ensure availability and sustainable management of water and sanitation for all (UNICEF & WHO, 2023). According to a recent report by the United Nations Children's Fund and the World Health Organization (UNICEF/WHO, 2023), Approximately 2.2 billion people lack access to safe drinking water, 4.2 billion lack proper sanitation services, and 3 billion lack basic handwashing facilities worldwide.

Globally, about 296 million with unimproved water sources and 115 million drinking surface water. An estimated 545 million with unimproved sanitation services and 419 million practicing open defecation (WHO, 2023). The challenges with water impact negatively to human health ,fitness, productivity, and quality of life (Ochola *et al*., 2022; Gough *et al*., 2020). Since 2015, coverage of safely managed drinking water has increased from 69% to 73% while sanitation has increased from 49% to 57% (UNICEF & WHO, 2023). Water crisis is the most prominent public health issue globally and risks the population to waterborne diseases, poor health and reduced productivity (Prüss-Ustün *et al*., 2019; UNICEF/WHO, 2021). Global evidence shows that better WASH could prevent the deaths of 297,000 children aged under five each year globally (WHO/UNICEF, 2021) Three out of five people lacked safely managed drinking water in Africa in 2020 (UNICEF, 2021). In sub-Saharan Africa (SSA), 400 million people have to use surface water and some have to walk long distances taking over 30 minutes from home to get improved drinking water sources. Out of the 1.4 billion people practicing open defecation or using unimproved or shared facilities globally, half a billion are from the SSA region (UNICEF & WHO, 2023). WASH coverage in the SSA region appears to be low with urban coverage being better than that in rural areas; 7.75% of total deaths due to diarrheal diseases across SSA are attributed to unsafe WASH (Zerbo et al., 2021). Water scarcity significantly threatens human health, the environment, and global food supply *(*Bayu *et al*., 2020). Drinking unsafe water impairs gut health through illnesses such as EED, diarrhea, and intestinal parasites infestation. Untreated excreta contaminates groundwater's and surface waters used for drinking, irrigation, bathing and household purposes exposing humans to enteric pathogens and diseases (Wolf *et al*., 2023).

In 2022, Eastern and Southern Africa had the lowest access rates to basic water and sanitation facilities, with over 226 million people (47%) lacking basic water services, and 381 million people lacking access to basic sanitation. Rural areas face greater challenges than urban centers in access to WASH (WHO, 2023). Nine countries including Kenya, Angola, Democratic Republic of Congo, Ethiopia, Madagascar, Mozambique, Sudan, Tanzania, and Uganda account for 80% of the underserved people in these regions. Ethiopia has the highest number of people lacking access to WASH services at 61 million, followed by Uganda with 27 million and Tanzania with 24 million (UNICEF/WHO, 2023).

In Eastern Africa, only 27% of the population have access to safely managed drinking water sources, 29% have access to basic water sources, 20% have limited access to drinking water, and 15% have access to unimproved water sources. In comparison, 9% depend on ground/surface water for drinking (UNICEF, 2022). In Ethiopia, the number of people who need access to WASH services rose from 7.3 million in 2020 to 20.5 million in 2023. In South Sudan, 41% of the people lack access to adequate clean water for drinking. The situation is worse in Somalia where half of the population especially displaced families cannot access safe and sustainable drinking water (Piper *et al.*, 2017; UNICEF, 2022).

Kenya is classified as one of the earth's most water-scarce countries. Frequent severe weather cycles, depleting natural resources, and rapid population growth add pressure to the already limited water supply for drinking and domestic use (UNICEF/WHO, 2023). Approximately 59% percent of Kenyans have access to safe drinking water, and 29 percent have access to basic sanitation. However, 9.9 million people still drink water directly from contaminated surface water sources and an estimated five million people practice open defecation (KDHS, 2022). Only 25 percent have hand-washing facilities with soap and water at home (KDHS, 2022; USAID Kenya, 2023). Water insecurity, combined with a lack of sanitation and hygiene products and services, endangers public health by increasing the risk of intestinal diseases, undermines economic productivity, and perpetuates social inequalities (USAID Kenya, 2023; Wolf et al., 2018).

The literature highlights the risks of inadequate WASH to people, as it exposes them to pathogens, infections, and gut inflammation. Chronic exposure to these conditions significantly contributes to high rates of EED, diarrhea, intestinal parasite infestations, and inflammatory gut syndromes, all of which adversely affect nutritional status, most severely among children under five in developing countries (Ramlal *et al*., 2019; Wandera *et al*., 2022) . Evidence indicates that improved WASH could prevent approximately 297,000 deaths of children under five each year globally (UNICEF, 2022). Despite various interventions and considerable literature on WASH, there remains a gap in effectively implementing these strategies within Kenyan communities, as reflected by the low adoption of WASH practices, particularly in resource-limited rural areas such as Siaya County (KDHS, 2022).

2.2 Gut health among children under five years

According to WHO, 2023, gut health can be defined as a state of physical and mental well-being in the absence of Gastro-Intestinal Tract (GIT) complaints that require the consultation of a doctor, in the absence of indications of or risks for bowel disease and in the absence of confirmed bowel disease. Furthermore, it can also mean a healthy upper and lower GI including prevention and avoidance of the diseases (Bischoff, 2011). In Summary, gut health covers multiple positive aspects of the GI tract, such as the effective digestion and absorption of food, the absence of GIT illness, normal and stable intestinal microbiota, effective immune status and a state of well-being (Lazar *et al*., 2024; Modern *et al*., 2022; Valdes *et al*., 2018). The primary role of the gut is to absorb nutrients whilst minimizing passage of non-nutritious foreign substances and pathogens (Crane, 2019)

Chronic exposure of the GIT to environmental pathogens, bacterial infections and inflammations contributes to the ever-increasing prevalence of EED among children under five years; a subclinical complex inflammatory small intestine disorder. It results from chronic gut inflammation by pathogenic bacteria leading to impaired intestinal wall, flattened villi and microvilli, inflammatory cell infiltrate causing nutrients malabsorption, increased gut permeability and loss of gut barrier function (Modern *et al*., 2022; Owino *et al*., 2016). The impaired gut barrier enables microbial translocation across the damaged gut mucosa, which is thought to drive a chronic state of systemic immune activation as well as diverting energy and resources away from growth and towards chronic inflammation thus contributing to poor nutrition stratus among children under five years (Afolami *et al*., 2021; Faubion *et al*., 2016)

Literature suggests that EED is attracting attention across the world because of its suspected central role in impairing gut health among children under-five years with compromised access to WASH (Owino *et al*., 2016; Regassa *et al*., 2023; Tickell *et al*., 2022). The impaired gut health is the major cause of failure of both nutritional and WASH oriented interventions to significantly improve child nutrition outcomes among under-fives globally (DeBoer & Guerrant, 2019; Grembi *et al*., 2019; Null, 2018). Globally, the prevalence of EED ranges from 50-90% and it is on the rise, with the highest cases in Asia and Sub-Saharan Africa (Chen *et al*., 2021). A case-control study in Peru, the United States of America (USA) and Zambia among 131 children between the ages of 2 to 36 months showed an EED prevalence of 53%, with LRR of 0.75, 5% with LRR of 0.14 and 82% with LRR of 2.26 respectively. It further indicated a significant difference in LRR between Peruvian and Zambian cohorts compared to US cohort ($p<0.001$) in terms of LRR

(Faubion *et al*., 2016). The findings indicated that the prevalence of EED was higher among developing countries, which could be attributed to poor WASH conditions and ever-growing populations.

Similar trends were also reflected in a study done in rural Malawi among 798 children between the age of 12 to 61 months indicated a high burden of EED among the children at 83 % (668), with 17 % (134) being severe and 66 % (524) having Moderate EED (Ordiz et al., 2016). Further studies in other rural Malawi set ups among 340 children 2-5 years and 400 children 12-59 months revealed an EED prevalence of 88% and 80.7% respectively (Semba et al., 2016; Benzoni et al 2015). In Ethiopia, an EED prevalence of 50% was noted among children 12-16 months (Chen et al., 2021). All these studies used the LR dual sugar tests for EED diagnosis. These are extremely high prevalence's of EED, it could be an indicator of poor gut health among under-five children and poor WASH conditions within the rural environment where the children exist.

In contrast, a study done in Tanzania showed a lower EED burden of 9% compared to the statistics reported by the Malawian study (Modern *et al*., 2022; Ordiz *et al*., 2016). However, a study in Gambia rural among 3 to 9 months' children showed an extremely higher EED prevalence of 95% compared to previously reported studies; similar value was reported in Malawi among children age 1-3 years (van der Merwe *et al.*, 2013; Yyan *et al*., 2014), which further reflects the burden of EED in the third world countries (Chen *et al*,.2021). In Kenya, a study in Kilifi County among under-fives showed an EED prevalence of 67% with a lactulose Mannitol ratio of more than 0.07 (Crane, 2019). The current trend of rising cases of EED, especially in resource limited setting in the developing countries necessitates the need to determine the overall effect of EED on nutritional status of children. From the literature it's evident that the prevalence of poor gut health is rising globally, with worse statistics in Africa. However, there is a gap knowledge on gut health status and malnutrition among children both in Kenya and Siaya County, thus need for the current study.

The EED among children can be determined using both the Dual Sugar test including the lactulose and rhamnose/mannitol (Disaccharide and monosaccharide sugars) and the ^{13}Carbon Sucrose Breath Test (^{13}CSBT). They are both stable isotope techniques providing a non-invasive diagnostic alternative to biopsy in determination of gut health problems among children and adults in both clinical and community set ups. To date, the most frequently used tests for EED are the dual sugar absorption tests. The most commonly implemented of these at present is the lactulose: mannitol (L: M) test or the L:R test, although other sugars such as xylose can be used. Lactulose is a large sugar that is not normally absorbed by the small intestine. Mannitol or rhamnose is a smaller sugar that is absorbed by the small intestine in proportion to absorptive surface area. In the L: M or L: R tests, after oral ingestion, both lactulose and mannitol/rhamnose are excreted intact in the urine following minimal metabolism.

Urinary mannitol/rhamnose therefore gives an index of absorptive capacity, while the presence of lactulose in the urine indicates impaired gut barrier function. Urinary excretion of disaccharides (lactulose) and monosaccharides (rhamnose,mannitol) and ratio of their excretion is a basis for measurement of intestinal permeability/gut barrier function(Mishra & Makharia, 2012). Higher urinary L:M or L:R ratios reflect greater abnormalities of one or both functions (Afolami *et al*., 2021; Crane, 2019; Ordiz *et al*., 2016). For the current study, L:R was used as a dual sugar test as per the Faubion *et al*.,(

2016) guidelines. Measurement of lactulose and Rhamnose in urine can be performed by enzyme linked immunosorbent assay, anion exchange chromatography, or mass spectrometry. All of these methods require centralized laboratory equipment and expertise, and the results are not always comparable between laboratories

The novel ^{13}C-SBT serves as a simple, non-invasive method for assessing intestinal absorptive function in humans by measuring total intestinal sucrase activity (Terry *et al*., 2012). This test is based on the release of $^{13}CO_2$ from ^{13}C-labeled substrates, such as ^{13}C-sucrose, which is used in non-invasive assessments of gut function, including tests for gastric emptying and gastrointestinal transit. In the ^{13}C-SBT, the breakdown of ingested 13C-sucrose by the sucrase enzyme produces glucose and fructose. After these metabolites pass through the liver and respiratory systems, $^{13}CO_2$ is released in the breath. By quantifying this $^{13}CO_2$, the test determines gut function using the Cumulative Percent Dose Recovery (cPDR) rate, following the protocol by Lee *et al*., (2020).

The use of highly enriched ^{13}C-sucrose as a breath test substrate enables a simple and sensitive, non-invasive assessment of small intestinal sucrase activity. It is also a faster and less labour-intensive method of assessing sucrase activity than the *in vitro* sucrase assay. A diminished level of small intestinal sucrase, for example due to damage or disease, would result in lower levels of breath $^{13}CO_2$ following ^{13}C-sucrose ingestion. The process of $^{13}CO^2$ production is illustrated in Figure 2.1

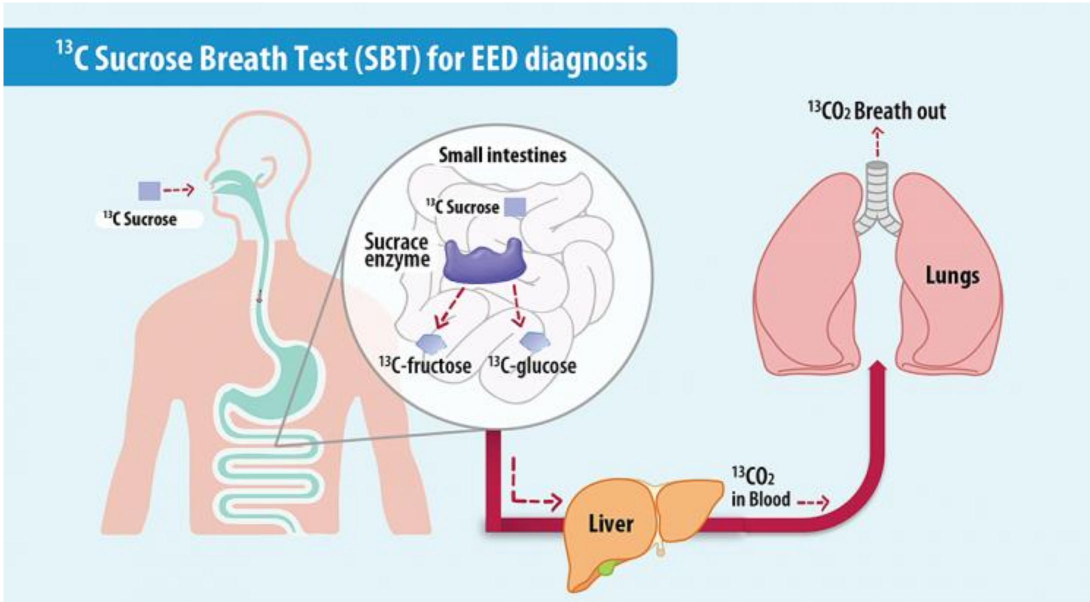

Figure 2.1: **Illustration 13 carbon breath test for diagnosis of EED.**

Source: *Schillinger et al, (2022)*

2.3 Nutrition status of children under-five years

The nutritional status of children below the age of five years is a key determinant of their general future health and productivity. Over the past three decades, undernutrition has become a major cause of morbidity and mortality among children under five years globally (Picbougoum *et al*., 2023; Wolf *et al*., 2023). In 2022, around 148.1 million children under five globally (22.3%) were stunted, meaning they were too short for their age, affecting more than one in five children worldwide. Additionally, 45 million children were wasted, being too thin for their height, while 37 million were overweight, indicating they were too heavy for their height (FAO, 2023). Over half (52%) of the global burden of stunting among under-five children were found in in Asia while 43% were in Africa. More than three-quarters of all children with severe wasting live in Asia and another 22 percent live in Africa (WHO/UNICEF, 2021)

SSA is home to some of the most nutritionally insecure people in the world (FAO, 2023). An analysis of demographic and health surveys on nutrition status of children under-five years in 32 SSA countries established that the pooled prevalence of wasting, underweight, and stunting were 8.1%, 17.0%, and 31.3%, respectively. Niger had the highest prevalence of wasting (21.5%) and underweight (37.1%), whereas Burundi had the highest prevalence of stunting (51.7%) among under-fives (Aboagye *et al*., 2022). A narrative review among SSA also showed a high prevalence of stunting among under-fives at 41% and was found to be higher in children above one year of age (Quamme & Iversen, 2022). In Uganda, stunting has been shown to be high over time, with a study reporting 40% of children under five being stunted (Ekholuenetale *et al*., 2020). Similarly, a study in Burkina Faso also showed high prevalence of stunting at 40.1% of the children, wasting in 25.1%, and underweight in 34% of the under-fives with stunting and underweight being associated with the sex (Picbougoum *et al*., 2023). The economic loss attributable to malnutrition has been estimated to be US$ 3.5 trillion annually, equal to 11 percent of the gross domestic product (GDP) of Africa and Asia (FAO, 2023)

In East Africa, 30.6% of children under five are stunted, and 5% are wasted (UNICEF/WHO, 2023). In Kenya, stunting stands at 18%, wasting at 5%, and underweight at 10%. Despite these numbers, malnutrition may not have improved, as the population base has grown between 2014 and 2022 (KDHS, 2022). In Siaya County, stunting is slightly higher than the national average at 19%, while wasting (2%) and underweight (7%) are below national levels (KDHS, 2022; KDHS, 2014). Wasting rates in East Africa and Kenya remain far from the World Health Assembly and SDG targets of below 5% by 2025 and below 3% by 2030, as rates appear to be stagnant or rising

(Quamme & Iversen, 2022; WHO, 2023). Stunting results in irreversible growth failure, with affected children unlikely to regain lost height or weight *(Habimana et al*., 2023). Poor sanitation, such as open defecation, is a key factor contributing to stunted growth in regions like India (Pastorelli *et al*., 2013).

Child stunting is a marker of chronic undernutrition and results from insufficient energy and nutrient uptakes coupled with chronic exposure to diseases (Koyuncu *et al.*, 2020). In addition, child wasting is the life-threatening result of poor nutrient intake and/or recurrent illnesses. Severe wasting among children weakens immunity and increases susceptibility to long-term developmental delays and an increased risk of death *(*Koyuncu *et al.*, 2020*;* Wandera *et al*., 2022). Children suffering from severe wasting require early detection and timely treatment and care to survive. Overall, children under five years are easily affected by mild and drastic changes in the environment where they live including environmental changes, hunger/drought, poor WASH, and diseases therefore, they require close monitoring and care to ensure optimal health and nutrition status (Ekholuenetale *et al*., 2020; FAO, 2023). The review shows limited progress in reducing undernutrition among under-five children, despite therapeutic, supplemental, and dietary interventions. This study aims to assess the impact of WASH on gut health and its role in undernutrition among children.

2.4 Water, sanitation and hygiene, gut health and nutrition status of under-fives.

The relationship between WASH, the gut health and nutrition status of children under five is deeply interconnected. Poor WASH conditions, such as contaminated water sources and inadequate sanitation facilities, contribute to the transmission of enteric pathogens (Sinharoy *et al*., 2021). These pathogens can lead to subclinical infections and chronic gut inflammation, which are key features of EED. The inflammation and structural damage to the intestinal lining impairs its ability to absorb nutrients effectively, contributing to malnutrition in children, studies have found significant relationship between EED and poor nutrition status with focus on linear growth failure (P<0.05) (Ghosh *et al*., 2021; Lin *et al*., 2020; Ritchie *et al*., 2009; Tickell *et al*., 2019). Another study found a significant association between wasting and handwashing before feeding (p=0.006) ,with lack of soap increasing the risks of diarrhea three fold (Tickell *et al*, 2021). This contributes to the burden of EED and continued poor nutrition status among children under five years.

Additionally, the consequences of EED extend beyond malnutrition, it is associated with alterations in the gut microbiota, which play a crucial role in nutrient metabolism and immune function (Tickell *et al.*, 2019; Valdes *et al*., 2018). Poor WASH conditions can disrupt the balance of beneficial and harmful bacteria in the gut, further exacerbating gut inflammation and dysfunction (Shrestha *et al*., 2020). The condition has been linked to stunting, a form of growth failure that affects physical and cognitive development. Stunting can have long-term effects on health and well-being, highlighting the importance of addressing WASH-related factors that contribute to EED development (Jamil *et al.*, 2021; Owino *et al*., 2016).

Improving hygiene practices, such as handwashing with soap, and ensuring access to clean water and proper sanitation facilities are essential steps in preventing EED and improving the gut health and nutritional status of children under five (Afolami *et al*., 2021; Sinharoy *et al.*, 2021). These interventions can help reduce the burden of EED and its associated health impacts on vulnerable populations. However, there is still much to understand on the linkage between WASH, gut health and nutrition status of children under five years

(Marie *et al.*, 2018; Tickell *et al*., 2019). Therefore, the current study focused on understand this complex relationship by assessing gut health and nutrition status among children with poor access to WASH in Siaya County Kenya.

2.5 Household, child and dietary factors influencing children nutrition status.

Household, child, and dietary factors, along with poor WASH conditions, contribute to the development of EED, a marker of poor gut health and declining nutritional status in children.

2.5.1 Household and child characteristics

Poor nutrition status among under-five children can be influenced by a variety of household and child-related factors. Families with limited income often struggle to afford a diverse and nutritious diet, contributing to nutritional inefficiencies that cause malnutrition among children. Additionally, food insecurity associated with poverty, conflicts, natural calamities, or climatic changes can contribute to inadequate nutrition for children in these households (Maingi *et al*., 2022).

Undernutrition outcomes like stunting, wasting and underweight have been shown to be significantly lower among children with mothers from high wealth status families compared to lower wealth status. The impact of unemployment and reduced income of household heads on the nutritional status of children aged below five years cannot be underrated. In Ethiopia, the prevalence of wasting, stunting, and underweight was higher among children aged 2-5 years of unemployed mothers than that of employed mothers (Zewdu & Handiso., 2020). Similarly, high education level was associated with improved nutrition status among under-fives ($p<0.05$) *(*Ekholuenetale *et al.*, 2020; Maingi *et al.*, 2022). The contribution of mother's education level to the inequalities in malnutrition among under-fives could be due to the differences in the knowledge or ability of mothers to decide proper nutrition for children in terms of balanced diets and accessibility to food commodities.

A study by Maingi *et al*.(2022) showed significant associations between stunting and the age of the mother (p=0.036) and education level (p=0.023). Education was also significantly associated with wasting and underweight at p=0.008 and p=0.014, respectively. Furthermore, the findings indicated that average household monthly income (p=0.019) and household economic activity engagement (p=0.01) influenced the nutritional status of children. These findings reiterate the role of socio-economic characteristics in determining the prevalence of malnutrition among children. Additionally, poverty, low education level and geographical locations determine the level of WASH conditions among households (FAO , 2023; Ghosh *et al*., 2021). This further, worsens the nutritional status of children by leading to diarrheal diseases and EED that again aggravates the already poor nutritional status among children. Thus, need to assess the role of household and child characteristics on rising cases of malnutrition among children under-five years in Siaya and Kenya.

2.5.2 Dietary intake and diversity among children under five years.

Children under five years are very sensitive to any slight change in dietary intake and diversity (Jamil *et al.*, 2021). Thus, the dietary intake of a child must supply the nutrients needed for their growth and development, and for body maintenance and energy for physical activities. Inadequate dietary intake causes nutritional. Poor nutritional status among under-fives, limit linear growth, inadequate muscle mass and lower their learning capacities, thus compromising their future, perpetuating an unending cycle of poverty and malnutrition with severe consequences to both individuals and nations (FAO/WHO, 2023). Inadequate dietary intake is secondary to insufficient or inappropriate food supplies causing food insecurity in the households. In some areas, cultural and religious food customs may affect dietary intake among children under five (UNICEF, 2023). Therefore, assessing the trends in dietary intake and diversification of diets among children helps in understanding the complexities of the underlying causes of malnutrition among children Siaya County and Kenya.

2.6 Summary of literature

The low adoption of WASH practices, particularly in resource-limited rural areas like Siaya County, Kenya represents a significant gap in addressing gut health and nutritional challenges with a focus on under-five children. Despite the known benefits of proper WASH practices in preventing gut diseases and improving nutritional status, the limited uptake in these areas highlights barriers such as lack of awareness, inadequate infrastructure, cultural practices, and insufficient resources. This gap not only hinders the prevention of waterborne diseases and malnutrition but also perpetuates the cycle of poor health outcomes among children. Addressing this gap is crucial to improving overall health and reducing the prevalence of gut conditions such as EED, which is strongly linked to poor WASH conditions. Further, a significant gap in knowledge and practices has been identified regarding the role of gut health status on persistent malnutrition levels among children under five years despite nutritional and WASH interventions. This gap necessitates the need for the current study to explore the relationship between gut health and malnutrition among children. This issue is compounded by the lack of an agreed non-invasive method for determining gut health and their respective cut-offs. While some studies have used LR, ^{13}CSBT, or conventional endoscopies and biopsies, no universally agreed diagnostic classification of EED exists. Disparities in findings among populations, especially in low-resource settings, persist. This study aimed to gain a better understanding of gut health among children 12 to 15 months and its link to their nutrition status using the L R test and the novel ^{13}CSBT to unravel the mystery of EED as a cause of sub-optimal gut health in children under five years in poor WASH communities in Siaya County, Kenya.

CHAPTER THREE: RESEARCH METHODOLOGY

3.1 Introduction

This section included details on the study area, population, and design, as well as the sampling technique and sample size determination. It also covers data collection, management, analysis, presentation, and ethical considerations.

3.2 Study area

The study was set in MCH Clinic of SCRH, in Siaya County, Kenya. Siaya County is located in the lake region, 65 km from Kisumu City. Siaya County borders Busia County to the North-West, Kakamega County to the Northeast, Vihiga, and Kisumu County to the East and Lake Victoria to the South. The longitude and latitudes coordinates of 0.0617° S and 34.2422° E. According to the 2019 population and housing survey, Siaya County is projected to have 1,059,458 people (KNBS, 2019). The county covers an approximate area of 2,530 Km^2 and has an estimated population density of 419 persons/km^2 with a 1.7% annual population change. The population is predominantly rural at 907,766 and urban at 85,417 people respectively. Children 0-9 years were 273,203 out of which about 171,228 were under the age of five years (KNBS, 2019). The major foods and cash crops grown in the area include maize, sorghum, millet, sugar cane, and subsistence livestock farming.

According to the Siaya County Integrated Development plan (CIPD), the area experiences water shortages and limited sanitation facilities, and aspects of open defecation are still reported in some areas (Siaya CIDP, 2023). The terrain exposes households to flush floods and sometimes stagnant water, contributing to poor WASH. The county reports high rates of poor WASH, with 51% and 65% lacking access to safe drinking water and basic sanitation respectively. About 44.2% lack pit latrines, while 19% practice open defecation (KDHS, 2022).

Siaya County faces several significant health challenges, with malaria being a leading cause of death, especially among infants, due to its high prevalence. The county also grapples with a high rate of HIV/AIDS, posing a major health concern. Respiratory infections, skin diseases, and diarrheal diseases are also common, contributing to the overall burden of illness. Additionally, severe malnutrition remains a widespread issue, further exacerbating the county's health crisis (KDHS, 2022).

Cases of undernutrition among under-fives are still high with stunting at 19%, wasting (2%), and underweight at 7%. The under-five mortality is 63 deaths per 1,000 live births in Siaya County compared to the national statistics of 41 deaths per 1000 live births (KDHS, 2022). Siaya County comprises about 173 health facilities under the Ministry of Health spread across the County. There is a doctor-to-population ratio of 1:44,000 against the recommended WHO ratio of 1 doctor to 1000 people (KDHS, 2022; WHO, 2023).

3.3 Study Population

The study involved children aged 12-15 months attending MCH clinic at SCRH. This range was selected because it falls within the window of infant growth faltering, which holds clinical and public health relevance, while also being old enough to minimize the influence of breastfeeding on LRR performance and to allow for a several-hour fast during the initial assessment of the test. These children are also highly exploratory, interacting with their surroundings, which increases their exposure to pathogens, soil, and fecal matter. A significant concern at this age also is teething, as children tend to put objects in their mouths to soothe discomfort. Since children under five years of age have an underdeveloped immune system, they are particularly vulnerable to diseases associated with poor WASH situation

3.3.1 Inclusion criteria

Only children who were between 12-15 months of age and permanent residents of Siaya County attending MCH clinic at SCRH, and whose caregivers accepted an informed consent form were involved.

3.3.2 Exclusion criteria

Children with illnesses/chronic conditions that may contribute to gut impairment and growth retardation were excluded from the study. This was done by scrutinizing hospital records and feedback from the caregivers.

3.4 Study design

The study adopted a cross-sectional analytical study design, which was suitable for examining the relationship between impaired gut health, as indicated by the presence or absence of EED, and the nutritional status of children aged 12-15 months at SCRH, Siaya County, Kenya. The study provided an assessment of the prevalence of EED, poor nutritional status, and inadequate WASH conditions within the study population.

3.5 Study variables

3.5.1 Dependent Variables

The dependent variable in this study was the nutritional status of children 12-15 months. The assessment outcomes were stunting (Height-for-Age Z-score), wasting (Weight-for-Height Z-score) and underweight (Weight- for- Age Z-scores) among the study children.

3.5.2 Independent variables

The independent variables were WASH practices (source of water, hand washing facilities, use of soap in hand washing, type of toilet and separate room as kitchen). Followed by determination gut health status measured and classified as either EED positive or negative depending on the diagnostic test outcomes among the study children. Lactulose and Rhamnose sugars ration (LRR) was primarily used determine gut health and followed up by the ^{13}CSBT as the novel diagnostic technique.

3.5.6 Intervening variables

Other variables would influence nutritional status among the children however, they were not the focus of the current study which included child characteristics like child age, child gender, and morbidity history, household socio-economic status e.g. income, education level of the mother, occupation, religion, number of household members and household head and dietary intake, diversity, and food security.

3.6 Sample size determination and sampling procedure

3.6.1 Sample size determination

Due to the limited data on the link between EED and undernutrition, a mean difference of at least +0.2 in height-for-Age Z-scores (HAZ) (stunting) between EED-positive and EED-negative groups was considered biologically relevant. It thus was considered in the determination of the sample size of the current study. For example, the Kenyan and Bangladesh WASH benefits study assumed a +0.15 difference in HAZ (Arnold *et al*., 2013). Furthermore, a statistical power above 80% is considered an adequate sample size to provide a significant difference in HAZ among the study population (Kirkwood & Sterne, 2003). Therefore, using the formula by Kirkwood & Sterne (2003), with a desired statistical power of 90% and a 5% significance level, the study required a sample size of at least 100 infants.

n =.$\frac{(u+v)^2(\delta_1^2+\delta_0^2)}{(\mu_1-\mu_0)^2}$……………………………………….. (Kirkwood & Sterne, 2003)

$\mu_1 - \mu_0$ – Expected mean difference in HAZ between EED positive and EED negative groups =0.2 (estimated from previous studies)

$\delta_1^2 + \delta_0^2$ = Standard deviations of HAZ estimates in the population (δ_1^2=0.5, δ_0^2=1.41)

u+v – constants proportions depending on desired level of significance (5%) and desired power of the study (90%) thus u=1.28 and v =1.96 (from the normal distribution table)

n= [$(1.28 +1.96)^2$ (0.5+ 1.41)]/0.2

=100 children

3.6.2 Sampling technique

Siaya County and SCRH were purposively chosen based on the predominant rural population catchment of 91.4% (907,766 people), prevalence of malnutrition and limited WASH facilities. Siaya County lacks adequate piped water and residents depend on water fetched from rivers, the Lake Victoria and boreholes exposing residents to gut diseases. The county has over 50% of the residents lacking access to safe drinking water. Diarrheal diseases and infections are also commonly reported in the County. Further, the county accounts for 19% of stunting, 2% wasting and 10% underweight among children (KDHS, 2022). Simple random sampling technique was used to recruit the sample population of children 12-15 months from those attending their regular MCH clinic at the SCRH hospital. A register of children attending the clinics was obtained. Using a computer-generated random number table, children were selected for the study and assessed during their regular clinic visits (Table 3.1).

Table 3.1: **Summary of sampling techniques**

Sample	Sampling technique
Siaya County	Purposively
Siaya County referral hospital	Purposive sampling
Children 12-15 years to participate in study	Simple random sampling

3.7 Data and information collection instruments

3.7.1 Semi-Structured Questionnaire

The questionnaires included child, household, and caretaker-related variables. The researchers captured the children's age, gender, breastfeeding status, dietary diversity, and food security status of the children. Further, looked at the socio-economic characteristics of the parents including education level, occupation, income, religion, household head, and number of household members. The researcher also collected data on the child’s household WASH characteristics. It involved probing the mother on the source of water for drinking and other purposes like cooking and washing. The questionnaire had various choices of water source including Piped into dwelling, piped to yard/plot, public tap/standpipe, and tube well or borehole, protected well unprotected well, and Surface water (river/dam). Type of toilet as Pit latrine without flush, flush to piped sewer system, flush to septic tank, and Flush to pit latrine. They were further asked to indicate if they have a separate room as a kitchen, handwashing station, and use of soap in handwashing.

The water and sanitation indicators like source of water for drinking and type of toilet were later classified as per the WHO, (2023) as either improved which is protected from contamination e.g. piped water, borehole, protected spring and collected rainwater or unimproved which is exposed to contamination e.g. unprotected well, surface water and water from vendors. Dietary intake and diversity were obtained a 24-hour recall was conducted on parents/caregivers relating to the types, frequencies, and number of meals given to children including continuous breastfeeding. This is to enable the determination of dietary diversity scores and food security status among the children. Dietary diversity score was classified as per the number of food groups consumed, high (> 6 food groups moderate (4-5 food groups), and low (> 6 food groups) while food security assessment was done by determining the number of food groups consumed whereby children having < 4 food groups were termed as food insecure (Gina & Ballard, 2010).

3.7.2 Anthropometric Assessment Form.

Information on nutritional status of children was collected following the anthropometric assessment including height (cm) and weight (kg) of the children. Height was taken using the horizontal SECA length board while weight was taken using the SECA digital weighing scale. The age of the child was determined before taking any anthropometric measurement to ensure that they are within the target age of 12-15 months. The correct age of the children was confirmed using their MCH handbook or by the response from the mother if the MCH handbook was not available. Height and weight measurements were taken and recorded in the semi-structured questionnaire anthropometric form section. Before use the weight scales were calibrated and after every weight taken the weighing scale was checked for conformity. The weights and heights were taken three times and their average done to ensure accuracy.

3.7.3 Urine and breath collection instrument

The information on LRR and ^{13}CSBT was captured in this form. It included the points of sample collections, codes and instruction for sample collection. The equipment needed for carrying out ^{13}CSBT were Labco Exetainers (evacuated tubes) from Labco Limited in the United Kingdom (https://www.labco.co.uk/products/breath-vials/product/383-exetainer-12ml-coated-evac-labelled-x-1000/category_pathway-19). These clear glass vials are unidirectional and allows breath to enter and cannot flow back. It helped in collection and storage of breath awaiting shipping for analysis and quantification of $^{13}CO_2$ in the breath. The tube was placed on the nose or the mouth of the child and breath captured as they breath in and out (**Figure** 3.1). Face masks were also available to protect the children from any infectious condition or flue from the research assistants. Bottled water was stocked for reconstition of $^{13}C_{12}$-sucrose in the ^{13}CSBT**.** There was also 0.5 g of 13 carbon sucrose sugars acquired the Cambridge Isotopes Company-United Kingdom (UK). It was covered in an airtight zip lock bag to prevent exposure to the air since it can absorb water from the atmosphere.

For LR test the study required the Lactulose and L-rhamnose sugars which were supplied by Xi'an Henrikang Biotech Company Limited from China. They were covered in airtight zip lock bags because they are anhydrous in nature and can absorb moisture from the atmosphere and solidify reducing their effectiveness and shelf life. The urine bags were obtained locally and were used to collect urine samples from the children. They were strategically positioned on the child’s genitalia to ensure that there were no spill overs of urine. Pumpers were also procured locally to aid in holding the urine bag in place and reducing risk of cross contamination with faecal matter, cotton wool for straining the urine into the container using syringe and 50 ml container, which was used to store the urine awaiting shipping to the laboratory for LR Test.

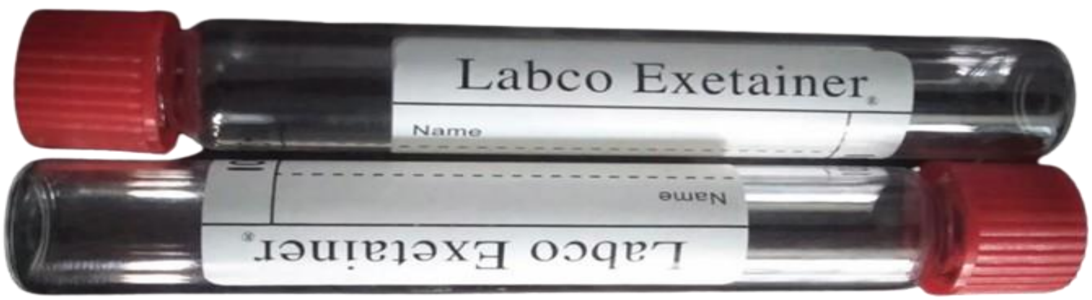

Figure 3.1: **Labco unidirectional exetainers for breathe collection**

3.8 Data and information collection procedures

The data collection process involved recruiting and training of research assistants, enrolling study participants and capturing of data as per the study objectives. Several instruments were employed in the study including structured questionnaires, anthropometric instruments and urine and breathe collection forms.

3.8.1 Training of research assistants

The researcher recruited four research assistants that included three qualified and licensed nutritionists who helped in baseline data collection and anthropometrics and one nurse who was key in clinical identification of children with various diseases conditions to check if they met the criteria for recruitment and supported the dosing process of the children. The recruitment was based on their ability to communicate with the study participants in *Dholuo*, Kiswahili and English and if they were conversant with the study area. They were trained for two days on the study objectives, data collections tools and data collection process.

The first day of training involved introduction of the research team, the study objectives and overview. Familiarization with the semi-structured questionnaire and each requirement was also done. The second day was training on sample collection for LRR test and ^{13}CSBT Test as outlined in Appendix III and IV. The research assistants were taken through a practical demonstration (role-play) on recruiting study participants, questionnaire administration and anthropometric measurements. Further, they practically demonstrated the process of urine collection using urine bag, and breathe collection using the labco exetainers tube on a dummy baby based on the protocol. The research assistants were trained on the use of various personal protective equipment, ensuring personal hygiene and self-care during the data collection period in the wake of COVID-19 pandemic.

3.8.2 Socio-demographic and economic data collection procedures

After identifying a potential participant, the research assistants introduced themselves to the mothers, explained the purpose of the study via informed consent and ensured the mothers were well-informed before participating in the study. Those who accepted and signed consent forms were taken through the structured questionnaire for socio-demographic and economic data.

The participant answered the questions using their preferred English, *Swahili,* or *Dholuo* translations. Back translation was used to ensure that meaning was preserved among the various languages used in probing for answers from the respondents. English was translated into *Swahili,* and the same was done for *Dholuo* to ascertain consistency in meaning and context. Questions asked included the name of the village, age and gender of the child, birth order number, number of household members, and education level of the mother, religion, source of income, average monthly income and marital status. In case any question needed to be clarified, clarity was sought from the respondents. The date of birth was also confirmed from the MCH booklet or hospital records in case of disparity.

3.8.3 Water, Sanitation and hygiene data collection procedures

After determining the sociodemographic characteristics, the research assistants further determined the WASH status of the households of children participating in the study. The mother was probed on questions relating to the source of water for drinking and other uses, whether it was from the household tap or public tap, from a protected well, rain water, from an unprotected well, a dam or surface water to determine if it was improved or unimproved source of water. The type of toilet was also chosen to indicate sanitation level, and it was classified as Pit latrine without flush, flush to piped sewer system, flush to septic tank and flush to pit latrine. They were further asked to indicate if they had a separate room as a kitchen, a handwashing station and use of soap in handwashing.

3.8.4 Anthropometric data collection procedures

The WHO, (2006) standards of anthropometric data collection were used in this study to collect data on the nutritional status. The height and weight of the children was taken and recorded in the anthropometric assessment form. This was used to compute the indices of nutritional status of Weight for Age Z-scores (WAZ), Height for Age Z-scores (HAZ) and Height for Age Z-scores (HAZ).

3.8.4.1 Height/length measurement

The body height was read to the nearest 0.1 cm for the study children able to stand. Those who were unable to stand on their own, a horizontal SECA-length board was used by laying it on a flat surface. The child's body length was taken without shoes with the child lying in a recumbent position. The height of children who can stand on their own was measured using a vertical wooden height board by placing the child on the measuring board, and the child standing upright in the middle of the board. The child was standing in an anatomical position with the back of the head, shoulders, buttocks, back of knees, and heels touching the board. Two measurements were taken for each child and averaged to the nearest 0.1 cm.

3.8.4.2 Weight Measurement

Weight measurements were conducted using a Salter scale for children unable to stand, with the child either sitting or lying flat. For children who could stand, a SECA digital scale was used. Each child's weight was recorded twice and averaged. Weights were measured with minimal clothing and no shoes. All weighing instruments were calibrated before the first measurement by setting them to zero, and subsequent calibrations were performed before weighing each child.

3.8.4.3 Classification of the children nutritional status indicators

The Nutritional status of children were classified according to the WHO, (2009) child growth standards as per Table 3.2

Table 3.2: **Classification of Nutritional status Indicators**

Nutritional status Indicator	Cut off	Term of status
Height for Age Z-scores (HAZ)	< -3 Z-scores	Severe stunting
	≥-3 to <-2 Z-Scores	Moderate stunting
	≥-2 Z-Scores	Normal
Weight for Height Z-Score (WHZ)	< -3 Z-scores	Severe Wasting
	≥-3 to <-2 Z-Scores	Moderate wasting
	≥-2 to < +2 Z-Scores	Normal
Weight for Age Z-scores (WAZ)	< -3 Z-scores	Severe underweight
	≥-3 to <-2 Z-Scores	Moderate underweight
	≥-2 Z-Scores	Normal
Nutrition status classification	HAZ or WAZ or WHZ >-2	Undernourished
	HAZ or WAZ or WHZ<-1	Normally nourished

3.8.5 Procedure for urine collection for lactulose-rhamnose ratio test

Experienced personnel carried out the collection of the urine sample for LR test which was used for the determination of children's gut health. First the sugars were reconstituted together to avoid dosing them differently, thus the ^{13}C-sucrose sugars and the dual sugars (LR) were mixed together. The procedure involved preparing 1 g lactulose and 0.2 g rhamnose and dissolving in 15 ml water (~300 mOsmol/L) as guided by Lee, *et al*, (2020) and Fabioun *et al*, (2016) studies, including the study protocol (Appendix III). Using a clean, sterile pipette, the appropriate volume of stock solution of ^{13}C-sucrose was transfered into the lactulose/rhamnose solution,each time a new pippete was used to prevent contamination while the used ones were taken for sterilization using the autoclaves in the laboratory at the SCRH. The reconstituted solution was stored in a freezer at a temperature of -20^{o}C to prevent mold growth on the sugars.

Lactulose, a large disaccharide, is minimally absorbed via the paracellular route and then excreted unchanged in the urine unless the permeability is altered. Altered intestinal permeability was defined as a ratio of urinary recovery of lactulose to rhamnose (L:R) > or =0.072 or based on the median of the L:R ratio (Fabioun *et al*, 2016; Lee, *et al*,2020). The current study adopted the median as the cut off for classifying the gut status based of L:R ratio which was 1.69 as proposed by Lee et al, (2020). Any ratio equal to or above 1.69 was classified as EED positive meaning there was higher gut damage increasing permeability of complex sugars like lactulose.

The procedure dictated that the child must fast atleast one hour before the test to reduce chances of sugars from foods taken influencing the outcome of the study tests. Baseline sample was taken from the child before dose administration. The child was encouraged to take water during the process of sample collection. The test involved administration of an oral dose of both lactulose sugar a 1g/kg body weight and rhamnose sugar at 0.2mg/kg body weight (Livingston, 2013). If the child spat out, vomited, or failed to swallow all the sugar solution, the test could not be completed. It was stopped and re-scheduled for another day. After baseline sample, collection of the subsequent samples continued while encouraging the child to drink water throughout the test.The sequential urine sample collection continued for 90 minutes.

3.8.6 Breathe sample collection procedure for 13- carbon Sucrose breath test

The ^{13}CSBT used a naturally enriched sucrose solution as the test substrate. The breath sample from the test was used to determine children's gut health status whether EED positive or EED negative.

Sucrose was broken down by sucrose enzyme located in the brush border of the small intestinal mucosa. Sucrase splits ^{13}C-sucrose into ^{13}C-glucose and ^{13C}-fructose. These sugars were absorbed through the mucosal cells and were metabolized by the liver, releasing ^{13}CO2. Labeled as bicarbonate, it was transported by blood to the lungs, where it was expelled in the breath. The release of ^{13}CO2 in the breath correlated with the amount of sucrase activity in the small intestine. If sucrase activity was diminished, it was a strong indicator that the epithelial cells lining the villi were damaged (Lee *et al.,* 2020; Ritchie *et al.,* 2009)

The procedure for ^{13}CSBT involved labeling four exetainer tubes in an appropriate rack coded with a number and alphabet identifier representing the study code, site code, participant code, timepoint, and replicate identifier (e.g. 1 SBT- T60-A). The child was allowed to settle in the MCH unit and adjust to study surrounding. This included even allowing the child to play with the breath sampling equipment. The children were fasted for one hour prior to the test. The first (baseline) sample was collected immediately before administration of the ^{13}C-sucrose solution. The Labco evacuated exetainers were directly used to collect breath because of its unidirectional property that enabled breath retention as a white precipitate in the glass tube. It involved placing the opened exetainers glass tube on the nostrils or mouth of the child for air to enter (some white precipitation was noticed on the glass tube). The choice of mouth or nostrils depended on whether the child had common cold/runny nose. After enough breath was collected, the tube was tightly capped and put on the rack.

After collection of the baseline breathe sample, ^{13}C sucrose solution was administered, the tube washed with 5ml water, and given to child. Continued to collection breath samples as outlined (breath was collected at baseline (T0), after 30(T2) minutes, 60(T3) minutes and 90 minutes (T4)). This was a modified protocol from the original that required breath sample collection after every 15 minutes for 90 minutes and after 30 minutes for the next 240 to 300 minutes. This was occasioned by the need to reduce turnaround time due to the emergence of COVID 19 pandemic at a time when the study was kicking off, as a precautionary measure in reducing chances of corona virus spread as reported by Jaika *et al*, (2024) paper on challenges in the application of stable isotopes techniques in the diagnosis of EED amidst the COVID-19 pandemic.

Breath samples were stored at room temperature in a box. Samples were stable up to at least six months before shipment was finalized. Indication of test failure included spitting or vomiting the test solution, child withdrawn from the study before 90 minutes,failure in collection of two consecutive breath samples prior to 90 minutes and breach of exclusion criteria identified retrospectively (Appendix III:Comprehensive Procedure for gut assesment). Breath samples were analyzed for $^{13}CO_2$ concentration using an isotope ratio mass spectrometer as outlined by Abimosleh, Tran & Howarth, (2013). In the laboratory, the samples were introduced into the analyzer, strategically positioned to ensure the all the $^{13}CO_2$in the breath was detected and quantified. The amount of $^{13}CO_2$ recovered from the breath was recorded in the excel sheet and simplified to enable other statistical analysis and inferences.

The ^{13}C-SBT was used to reliably measure the degree of small intestinal mucosal damage among asymptomatic children with an underlying EED thus determining the integrity and absorptive capacity of the small intestine (Ritchie *et al*, 2009). The 13C-SBT was an attractive option in community settings due to its ease of administration and the noninvasive nature. However, accounting for the effects of diarrhea and other intestinal conditions on the test outcomes remained challenging

3.9. Validity and reliability of the research instruments

3.9.1 Validity

The researcher used face and content validity to measure the degree to which data collected using the questionnaire instrument represents. The researcher involved experts from the department of nutritional sciences to evaluate the content of the questionnaire and determine if it meets the objective. Any question that needed clarity or modification was done as recommendations. For example, there was need to clarify on questions related to WASH for specificity and questions aimed at probing history of morbidities among children were to be made more specific for easier understanding by the caregiver.

3.9.2 Reliability

The ^{13}C-SBT and the LR test had already undergone optimization by experts in relation to effectiveness in the diagnosis of EED. Therefore, the researcher was guided by the protocol published by Lee, *et al*. (2020) and Faubion *et al*. (2016). The cost of the sample collection and the requirements did not allow for repeat of test for reliability in the study. There were shortages in supply of equipment needed for the study worsened by the emergence of COVID-19 pandemic and strict protocols were to be followed. The approvals for the pretest study in a different region with similar characteristics proved to be a challenge to the study team.

3.9.3 Quality assurance

The researcher assessed the semi-structured questionnaire for validity before the actual data collection to ensure they addressed the specific objectives of the study. The research assistants recruited in the study were licensed nutritionists and nurses with at least a diploma in nutrition and dietetics or nursing. The research assistants were fluent in the three languages used in the study area including English, *Swahili* and *Dholuo*. The research questions were through the back translation from English to Swahili and back to English for quality control measure to determine if the meaning was the same. Similar process was done for *Dholuo* language. The original tool was in English; the translation depended on the preferred language by the respondent. The research assistants were trained on the objectives of the study, the data collection tools and procedures, and were closely supervised by the researcher during data and sample collection process. The researcher screened the questionnaires for completeness and assessed the samples if they met threshold on a daily basis and legibility before accepting them from the research assistants. All data was cleaned using frequencies and missing values, invalid entries were counter-checked with the responses on the questionnaires and samples for accuracy, and where necessary the respondents or research assistants were contacted for clarification

3.10 Data analysis and management

3.10.1 Data management

The data entry format allowed for immediate data checks as filled by data collectors and the research assistants. The principal investigator did additional data quality control throughout data collection process. Laboratory data was batched and merged in the database as they became available.

3.10.2 Data analysis

The cleaned data was entered into the Statistical Package for Social Sciences (SPSS) version 26. Anthropometric indices were calculated using WHO Anthro software (Version 3.2.2, 2006), with a -2SD cut-off to classify children as stunted based on HAZ, wasted based on WHZ,or underweight based on WAZ according to WHO,(2006) standards. Descriptive statistics, including percentages and means, were used to analyze trends related to objectives one and two. T-test and one-way ANOVA were used to determine differences between study variables. Chi-square tests and Pearson correlation analyzed associations among categorical variables, while simple linear regression assessed the strength relationships between WASH indicators, gut health, and children's nutritional as highlighted in Table 3.3.

Table 3.3: **Summary of data analysis and presentation**

Objective	**Data analysis**	**Data presentation**
To assess water, sanitation and hygiene situation among children aged 12-15 months	Descriptive statistics; percentages, Chi-square test	Tables
To determine the gut health of children aged 12-15 months.	Descriptive statistics; percentages; chi-square, t-test	Bar graphs, Tables
To determine the nutrition status of children 12-15 months	Descriptive statistics; percentages; Z score	Tables, graph
To establish the relationship between WASH and gut health among children 12-15 months	Inferential statistics-Chi-square test, linear regression	Tables
To establish the relationship between gut health and nutrition status among children aged 12-15 months	Inferential statistics-chi-square test, ANOVA, linear regression	Tables

3.11 Ethical and Logistical Considerations.

Ethical approval was sought from Masinde Muliro University of Science and Technology, Institutional Ethics and Research Committee (MMUST-IERC/012/2022- Appendix VII). Further, a permit from National commission of Science, Technology and Innovation (NACOSTI/P/21/13254- Appendix VIII) was obtained. Permission was also sought from the Ministry of Health, Siaya County and the hospital administration (Appendix XI). Informed Consent was sort from the participating mothers after explanation of all the research procedures and requirements. All mothers and infants requiring medical care were referred to the SCRH. All cases of unforeseen effects because of the procedures used was also referred. No adverse effects were expected because of the research procedures. A summary of ethical considerations is in Appendix V.

CHAPTER FOUR: RESULTS

4.1 Introduction

This chapter presents the study findings. The purpose of the study was to determine the linkage between gut health and nutrition status among children aged 12-15 months with poor access to WASH attending MCH clinic at SCRH, Siaya County, Kenya. The presentation of data is in line with the study objectives. The study involved a sample size of 100 children representing a response rate of 100%. The issued questionnaires were filled completely by the respondents according to the directives given by the researcher.

4.2 Socio-demographic and economic characteristics of respondents and children

4.2.1 Socio-demographic characteristics of respondents

Table 4.1 indicates that the majority of respondents were married (80%), while 19% were single, and 1% were widowed. Most respondents (96%) were mothers to their children, with the remaining 4% serving as caregivers. The mean age and weight of respondents were 28 years and 62.34 kg, respectively. Regarding religious affiliation, 58% identified as Protestant, 29% as Catholic, and 13% as adherents of African traditional religions.

Educationally, 40% of respondents had completed primary education, 42% had secondary education, and 18% had attained tertiary-level education. Economically, most respondents (69%) reported a monthly income between Ksh. 1,000 and 10,000, while 24% earned between Ksh. 10,001 and 20,000, and 6% reported incomes of Ksh. 20,001 to 30,000. Only 1% earned Ksh. 40,000 or more. At the time of data collection, 6% of mothers were pregnant. The household's houses had a mean of two rooms, with a mean of four individuals sleeping per house. In terms of assets, 54% of households owned a television, 91% had a table, 9% owned a refrigerator, and 91% had a mosquito net.

Table 4.1: **Some socio-demographic characteristics of the respondents**

Socio-demographic characteristics	**Response**	**n**	**%**
Marital status	Married	80	80.0
	Single	19	19.0
	Widow	1	1.0
Relationship to the child	Mother	96	96.0
	Caregiver	4	4.0
Mothers age	Mean (years)	28 ±6	
mothers weight	Mean (Kg)	62.3±10.6	
Respondent's Religion	Catholic	29	29.0
	Protestant	58	58.0
	African religion	13	13.0
Education level of respondents	Primary	40	40.0
	Secondary	42	42.0
	Tertiary	18	18.0
Mothers monthly income (Ksh.)	1,000-10,000	69	69.0
	10,001-20,000	24	24.0
	20,001-30,000	6	6.0
	40,001-50,000	1	1.0
Is the mother currently pregnant?	Yes	6	6.0
	No	94	94.0
No. of people sleep in household	Mean	4 ± 2	
No. of rooms in the house	Mean	2 ±1	
The household own a television	Yes	54	54.0
	No	56	56.0
The household own a table	Yes	91	91.0
	No	9	9.0
The household owns a refrigerator	Yes	9	9.0
	No	91	91.0
Use of mosquito treated nets	Yes	91	91.0
	No	9	9.0

4.2.2 Some child demographic and morbidity characteristics

From the sample size of 100 children involved in the study, 54.0% were male and 46.0% female. The mean age of children in the study was 14±1.5 months, with a range of between 12 and 15 months. The mean birth weight of the children was 3.17 kg (1.7- 4.7kg). In addition, 87% of the children were reported to have good appetite, with 13% having poor appetite. The prevalence of diarrhea was at 36% among children in the past four weeks. Among the children, 13% were reported to have had mucus/blood in the stool while worms were observed in stools of 14% of the study children. Slightly over half of the mothers reported to have treated their children for worms infestation in the last six months while 56% reporting regular deworming of their children (Table 4.2).

***Table 4.2**:* Demographic and morbidity characteristics of children

Child characteristics	**n**	**%**
Gender of the children		
Male	54.0	54.0
Female	46.0	46.0
Mean Age of children(Months)	14±1.5	
Mean birth weight	3.17(1.7- 4.7)	
Child appetite test		
Poor	13	13.0
Good	87	87.0
Diarrhea over past 4 weeks	36	36.0
Blood/mucus observed during diarrhea	13	13
Observed Worms in child’s stools	14	14.0
Worm infestation treatment (last 6 months)	53	53.0
Regular child deworming	56	56.0

4.2.3 Some dietary and child feeding practices

Regarding dietary intake, the children consumed a mean of 5 ± 2.186 meals in the previous 24 hours. Over half of the children (60%) were breastfed, with a mean number of breastfeeding sessions of 3.6 ± 2.19 times. Most (93%) of the children consumed other types of milk, such as fresh milk or milk powder, within the past 24 hours. Additionally, more than half (62%) had eaten sugar cane or cane-derived foods, 61% consumed maize, 74% ate sorghum, and 68% consumed millet. Furthermore, 80% reported consuming rice or noodles, 55% ate yams or potatoes, 11% ate carrots or yellow/orange-fleshed vegetables, and 83% consumed dark green vegetables. Foods made from beans, peas, or corn were consumed by 37%, mangoes, papayas, or other sweet yellow-fleshed fruits by 37%, and other fruits or vegetables by 73%. Additionally, 13% ate meat, 29% consumed eggs, 37% ate fish, 25% consumed dairy products, and 24% had sugary foods like cakes. The estimated Dietary Diversity Score (DDS) showed a mean DDS of 4 ± 1.34. Of the children, 14% had a high DDS, close to half (49%) had a moderate DDS, and 37% had a low DDS. In terms of food security, findings indicated that over half (64%) of the children were food-secure (Table 4.3).

Table 4.3: **Dietary and child feeding practices**

Dietary and child feeding practices	**n**	**%**
Mean number of meals ,last 24 hours	5± 2.186	
Continued breastfeeding	60	60.0
Mean number. times breast fed ,last 24 hours	3.6± 2.19	
Other milks like fresh milk, Milk powder, last 24 hours	93	93.0
Intake of sugarcane, cane derived or combination	62	62.0
Intake maize or in combination of other foods	61	61.0
Sorghum intake or in combination of other foods*	74	74.0
Millet intake or in combination of other foods	68	68.0
Rice, porridge, noodles, other foods made from grain	80	80.0
White potatoes, yams or other foods made from roots	55	55.0
Carrots, sweet potatoes ,yellow or orange inside	11	11.0
Any other dark green vegetables e.g. spinach	83	83.0
Foods made from beans, peas, corn, groundnuts	37	37.0
Ripe mangoes, papayas or sweet yellow/orange/ red fruit	37	37.0
other fruits, vegetables(banana, apple, orange, avocado, tomato)	73	73.0
Any meat e.g. chicken, beef, lamb, goat or duck	13	13.0
Eggs	29	29.0
Fresh/dried fish	37	37.0
Dairy products e.g. mala, yoghurt or other dairy	25	25.0
Any sugary foods e.g. pastries, cakes or biscuits	48	48.0
Food secure (> 4 food groups)	64	64
Mean Dietary Diversity score(DDs)	4±1.34	
High DDs (> 6 food groups)	14	14
Moderate DDs (4-5 food groups)	49	49
Low DDS (1-3 food groups)	37	37

Note. N= 100, *other than maize, sorghum, millet, DDs- Dietary Diversity scores

4.3 Water, sanitation and hygiene situation among children

In the study, the majority of households (41.0%) reported using public taps or standpipes as their primary water source, followed by 22.0% who relied on surface water sources, such as rivers, dams, lakes, ponds, or streams. Additionally, 10.0% obtained water from boreholes or tube wells. Among those using boreholes or tube wells, 10.0% had protected sources, while 2.0% used unprotected ones. Only 3.0% of households had piped water directly into their dwellings.

For household water usage beyond drinking, 41.0% of households used public taps or standpipes for cooking, washing, and other domestic activities. Surface water was used by 21.0%, piped-to-yard or plot water by 13.0%, tube wells or boreholes by 11.0%, protected wells by 11.0%, and unprotected wells by 2.0%. Only 2.0% of households with piped water to the dwelling used it for additional household purposes. Overall, most households (76%) had access to improved drinking water sources, with 77% also using improved sources for other domestic needs.

Regarding sanitation, 90.0% of households used pit latrines without flush capabilities, while 6.0% had flush toilets connected to the sewer system, 2.0% flushed into septic tanks, and another 2.0% flushed into pit latrines. Overall, only 10% of households had access to improved toilet facilities, while 90% relied on unimproved sanitation options.

Concerning the environment for food preparation and storage, the study found that 62.0% of households did not have a separate room designated as a kitchen, while 38.0% did. Hygiene practices were assessed by examining the availability of handwashing stations. A majority of households (69.2%) lacked a functional handwashing station, while only 30.8% had one. Among those with handwashing stations, just 20% reported using soap for handwashing (Table.4.4).

Table 4.4: **Water, Sanitation and Hygiene indicators of households with under-fives.**

WASH indicators	n	%
Main source of drinking water		
Piped into dwelling	3	3.0
Piped to yard/plot	12	12.0
Public tap/stand pipe	41	41.0
Tube well or borehole	10	10.0
Protected well	10	10.0
Unprotected well	2	2.0
Surface water (river/dam etc.)	22	22.0
Overall drinking water status		
Improved(Piped water, protected well)	76	76.0
Unimproved (surface water, unprotected well)	24	24.0
Water source for other purpose e.g. cooking		
Piped into dwelling	1	1.0
Piped to plot	13	13.0
Public tap/stand pipe	41	41.0
Tube well or borehole	11	11.0
Protected well	11	11.0
Unprotected well	2	2.0
Surface water (river/dam.)	21	21.0
Overall status, water source for other purpose		
Improved (Piped water, protected well)	77	77.0
Unimproved (surface water, unprotected well)	23	23.0
Type of toilet facility		
Pit latrine without flush	90	90.0
Flush to piped sewer system	6	6.0
Flush to septic tank	2	2.0
Flush to pit latrine	2	2.0
Overall status of toilet facilities		
Improved (Flush to piped sewer system, septic tank)	10	10.0
Unimproved(Pit latrine without flush)	90	90.0
Handwashing station available (Yes)	31	30.8
Use of soap in handwashing (Yes)	20	20.0
Number of rooms in the house	Mean	2.39±1.67
Number of people sleeping in the house	Mean	4.19±1.49
Separate room as Kitchen (yes)	38	38.0

4.4 The gut health status among children

Gut health in children aged 12-15 months was assessed using the Dual Sugars LRR and the ^{13}CSBT to evaluate gut permeability. Based on the outcomes of these tests, children were classified as either EED positive or EED negative, with the presence or absence of EED serving as an indicator of their gut health status.

4.4.1 Lactulose/Rhamnose dual sugars test for gut permeability

The LRR served as the primary measure for determining gut health, classifying it as either EED positive or EED negative. According to Lee, *et al*.. (2020) protocol on EED classification, an LRR value above or below the cutoff of 1.69, the median value, was used for this classification. The study results showed that 53.0% of children aged 12-15 months tested positive for EED, while 47.0% tested negative as in Figure 4.1.

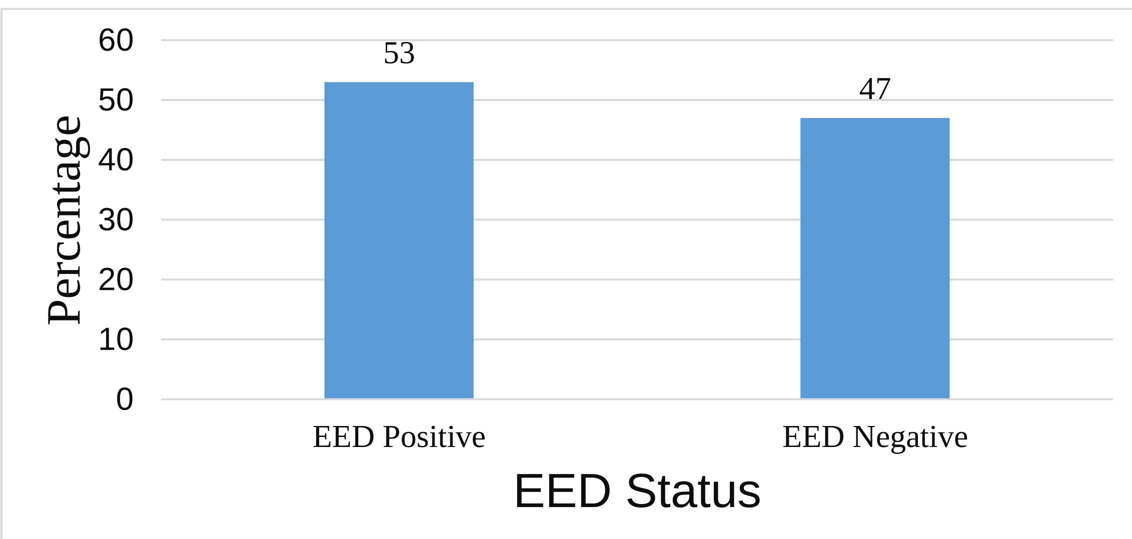

Figure 4.1: **EED status of children 12-15 months**

The mean urine lactulose recovery was 38.41 ± 42.23 µg/L (range: 1.44–300), and the mean rhamnose recovery was 29.99 ± 63.38 µg/L (0.94–589.12). The average LR ratio was 3.03 ± 4.32 (0.19–32.38). An independent t-test indicated a significant difference between EED-positive and EED-negative groups in both LRR ($t(98) = 4.79$, $p < 0.05$) and rhamnose recovery ($t(98) = -2.75$, $p < 0.007$) (Table 4.5).

Table 4.5: **Dual sugars outcomes and EED status**

Item	Mean (SD)	Min-max	t	df	p-value
Lactulose (ug/ml)	38.41(42.23)	1.44-300	1.36	98	0.176
Rhamnose (ug/ml)	29.99(63.38)	0.94-589.12	-2.75	98	**0.007***
LRR	3.03(4.32)	0.19-32.28	4.79	98	**0.000***

Note. Independent samples t-tests determined coefficient (t) values, with df-degrees of freedom. *Significant difference denoted at $p < 0.05$.

4.4.2 ^{13}Carbon sucrose breath test for gut permeability

The researcher measured the mean $^{13}CO2$ recovery at baseline (cPDR at baseline), at 30 minutes (T2), at 60 minutes (T3) and at 90 minutes (T4) post-dosage. The mean change in $^{13}CO2$ from baseline to 90 minutes was -0.45±1.96 (-0.842 to -0.063), indicating a low recovery rate of $^{13}CO2$ from breath. This result is consistent with the previous LR ratio results, where more than half of the children were EED positive, and indicated reduced sucrase activity due to gut mucosal damage. The mean time to peak was 51.33±16.64 minutes. The $^{13}CO2$ recovered at baseline (T0) was -14.20±2.39, while at 90 minutes (T4) it was -13.49±2.48. A paired sample t-test showed a significant difference in $^{13}CO2$ recovered at baseline and 90 minutes among the children in the study ($t(99) = -2.31$, $p = 0.023$) (Table 4.6).

Table 4.6: **Mean difference in $^{13}CO2$ at baseline and at 90 minutes**

Variables	**Mean(SD)**	**Mean diff.**	**95% CI of diff.**		**t (df)**	**P-value**
			upper	**lower**		
$^{13}CO_2$ at T0	-14.2(2.39)	0.45 (1.96)	-0.842	-0.063	-2.31(99)	**0.023**
$^{13}CO_2$ at T4	-13.7 (2.48)					

Note. Coefficient (t) was determined by paired samples t-test. $^{13}CO_2$ at T0 refers to baseline recovery, while $^{13}CO_2$ at T4 represents recovery at 90 minutes. Significance is indicated by $p < 0.05$.

4.4.3 Comparison between ^{13}CSBT and the LR ratio test parameters.

A Pearson correlation analysis showed no significant association between ^{13}C SBT cPDR at 90 minutes of recovery and the following variables: LR ratio ($r = 0.163$, $p = 0.105$), urine lactulose (μg/mL) ($r = -0.078$, $p = 0.443$), and rhamnose (μg/mL) ($r = 0.086$, $p = 0.395$) (Table 4.7).

Table 4.7: **Comparison between ^{13}CSBT and LR ratio**

	r	p-value
LR ratio	0.163	0.105
Lactulose (ug/ml)	-0.078	0.443
Rhamnose (ug/ml)	-0.086	0.395

Note. r represents the Pearson correlation coefficient. The independent variable is 13CSBT cPDR at 90 minutes. The association is significant *at p* < 0.05.

4.5 Nutritional status of children aged 12-15 months Siaya County, Kenya.

Height, weight and Mid-upper arm circumference were the anthropometric measurements used to determine the nutrition status of the children. The mean weight was 9.6 ±1.2 kg with a range of between 6.1 kg and 12.5kg and mean height of 80.9 ±5.4cm with a range of between 67.0 cm and 99.0cm. The mean Mid-Upper arm circumference was 15.6±0.60cm with a range of between 13.6cm and 17.4cm. The Z scores included Height-for-Age (HAZ), Weight-for-Age (WAZ), Weight –for-Height (WHZ) Z-scores. Generally, more than half of the children (52.0%) had some form of undernutrition (underweight, stunting or wasting) with 48% being normally nourished (Table 4.8).

4.5.1 Nutritional status by Weight-for-Height Z-scores

Majority of the children (55.0%) had normal WHZ, 29.0% were moderately wasted while 16.0% severely wasted. The results indicate a high prevalence of wasting (45%) among the children in the study area (Table 4.8).

4.5.2 Nutritional status by Weight- for- Age Z-scores

The study findings revealed that majority of children (86.0%) had normal WAZ (-1 to +2SD). Approximately 10.0% of the children were moderately underweight (-3SD to < -2SD) while 4% were severely underweight with a Z-score of <-3SD. These results indicate a generally high prevalence rate of underweight (14.0%) among the children. Upon chi-square analysis underweight indicated a significant association with EED status (p=0.027) (**Table 4.8).**

4.5.3 Nutritional status by Height- for- Age Z-Scores

The results showed that 92.0% of the children had a normal HAZ. Eight percent were stunted with 2% of the children being severely stunted (<-3SD), 6% moderately stunted and overall stunting was 8%. Chi square analysis showed a statistically insignificant association between stunting and gender among the children (p=0.21). The findings are illustrated in Table 4.8.

Table 4.8: **Children nutrition indicators by EED status**

	EED+ve		**EED-ve**		**Total**		**χ^2**	**p-value**
Nutritional indicators	**n**	**%**	**n**	**%**	**n**	**%**		
Weight-for-height								
Severely wasted (<-3 SD)	11	20.8	5	10.6	16	16	5.05	0.53
Moderately wasted(>-3 to -2SD)	15	28.3	14	29.8	29	29		
Normal (>-2 to +2 SD)	27	50.9	28	59.6	55	55		
Total	53	100	47	100	100	100		
Weight-for-Age								
Severely underweight (<-3 SD)	3	5.7	1	2.1	4	4	7.25	**0.02***
Mod. Underweight(>-3 to <-2)	9	16.9	1	2.1	10	10		
Normal (>-2 to +2 SD)	41	77.4	45	95.7	86	86		
Total	53	100	47	100	100	100		
Height-for- Age								
Severely stunted(<-3 SD)	1	1.9	1	2.1	2	2	0.48	0.79
Moderately stunted(>-3 to <-2SD)	4	7.5	2	4.3	6	6		
Normal (>-2SD)	48	90.7	44	93.6	92	92		
Total	53	100	47	100	100			
Overall nutrition status								
Undernourished	30	56.6	22	46.8	52	52	0.96	0.33
Normally nourished	23	43.4	25	53.2	48	48		
Total	**53**	**100**	**47**	**100**	**100**	**100**		

Note. *Significant at $p < 0.05$. $\chi 2$ = chi-square coefficient, SD = standard deviation, *EED +ve* = test positive for EED, *EED -ve* = test negative for EED.

4.6 Water, sanitation and hygiene, gut health and nutrition status among children

4.6.1 Water sanitation, hygiene, and gut health indicators among children.

Pearson correlation analysis revealed significant negative correlations between WASH indicators and gut health status. Soap use during handwashing (r (100) = -0.232, p = 0.020) and the availability of a handwashing station (r (100) = -0.218, p = 0.029) were negatively associated with the LR ratio. The availability of a handwashing station was also negatively correlated with lactulose recovered in urine (r (100) = -0.316, p = 0.001). However, no significant relationship was found between 13CSBT results and any WASH indicators (Table 4.9).

Table 4.9: **WASH indicators and gut health among children**

WASH Variables		LR	Lactulose	Rhamnose	13CSBT
Drinking water source	Coefficient(r)	-0.186	-0.008	0.029	-0.61
	P-value	0.296	0.936	0.777	0.545
Soap use in hand wash	Coefficient(r)	-0.232	-0.137	-0.048	0.019
	P-value	**0.020***	0.175	0.633	0.850
Hand washing station	Coefficient(r)	-0.218	-0.316	-0.207	0.075
	P-value	**0.029***	**0.001***	0.038	0.457
Type of toilet	Coefficient(r)	-0.026	-0.061	-0.029	-0.144
	P-value	0.797	0.546	0.774	0.158
Separate kitchen	Coefficient(r)	-0.124	-0.151	0.144	-0.18
	P-value	0.221	0.871	0.154	0.06

Note. *r was* determined by Pearson correlation coefficient. *Significant at $p < 0.05$.

The researcher further used a linear regression analysis to determine the strength of the relationship between WASH and gut health. The results showed statistically significant negative relationship between source of drinking water and gut health (*B*= -0.78, *p*=0.039, 95% CL**).** This may mean that one unit change in sanitation of water source leads 0.78 decrease in poor gut health among children. Therefore, the researcher rejects the null hypothesis that there is no significant relationship between WASH and gut health among the children and accepts the alternative (Table 4.10).

Table 4.10: **WASH indicators and gut health among children**

			95%CL		
Variable	**B**	**SE**	**LL**	**UL**	**p-value**
Main source of drinking water	-0.78	0.08	-0.344	-0.009	**0.039***
Type of toilet facility used	-0.16	0.09	0.031	-0.344	0.101
Use of hand washing soap	-0.04	0.17	-0.363	0.294	0.834
Handwashing station availability	-0.16	0.14	-0.447	0.122	0.259
Separate room as kitchen	-0.14	0.09	-0.319	0.039	0.124

Notes: Dependent variable: Gut health (determined by L: R ratio), *B* -regression coefficient, *significant at $p<0.05$

4.6.2 Gut health and nutrition status among children

Table 4.11 summarizes the relationship between gut health and nutrition status among children and odds ratio reported. Underweight showed a significant relationship with gut health, whereby improved gut health (Non-EED) contributed to 4 times reduction in underweight prevalence among children and vice versa (OR=3.663, 95% Cl: 0.750-3.413; $p<0.05$). However, wasting and stunting were not significantly predicted by being EED negative or positive among the study children.

Table 4.11: **Gut health and nutrition status among children**

Variable	**χ^2(df)**	**OR**	**95% CL**		**P-Value**
Wasting	1.897	1.600	0.750	3.413	0.168
Underweight	6.994	3.663	0.999	13.425	**0.008***
Stunting	0.052	1.104	0.457	2.666	0.820

Independent variable; Gut health (EED or Non EED*)*, χ^2-Pearson chi-square co-efficient, *****Significant at *p*<0.05, df=degree of freedom, OR-Odds ratio, Cl -Confidence interval

Further, the variation in nutrition status indicators between EED+ve and EED-ve children was determined. The results indicated a significant difference in child weight and WAZ among EED +ve and EED -ve children, $F(1, 98) = 5.291$, $p = 0.024$ and $F(1, 98) = 5.439$, $p = 0.022$ respectively. Other nutrition indicators including HAZ and WHZ did not indicate significant difference in means among EED positive and EED negative children (Table 4.12).

Table 4.12: **Nutritional status and EED status among children**

Variable	**Mean(SD)**		**95% C.I for mean**		**F(1,98)**	**P-value**
	EED +ve	**EED -ve**	**Lower**	**Upper**		
Child weight (kg)	9.32 (1.018)	9.82(1.239)	9.038	9.599	**5.291**	**0.024***
HAZ	2.92(.351)	2.90(.362)	2.783	2.990	0.149	0.214
WAZ	2.72(.568)	2.94(.323)	2.841	3.031	**5.439**	**0.022***
WHZ	2.30(.799)	2.49(.688)	2.288	2.691	1.562	0.701

Notes: *F*-statistic for ratio of variance in ANOVA, *Variation significant at *p*<0.05, *SD*=standard deviation; *HAZ*=Height-for-Age Z-scores, *WAZ*=Weight-for-Age, *WHZ*=Weight-for-Height Z-Scores

A further linear regression analysis between gut health (independent) and nutrition status (dependent) variables show no significant relationship (*p*>0.05) between poor gut health and nutrition status. As a result, the researcher fails to reject the null hypothesis and concludes that there is no significant relationship between gut health and nutrition status (Table 4.13).

Table 4.13: **Gut health and nutrition status of children**

			95%CI for B		
Variable	**B**	**SE**	**LL**	**UL**	**p-value**
HAZ	-0.114	0.536	-1.230	-1.179	0.950
WAZ	-0.312	0.282	-0.872	0.247	0.271
WHZ	-0.380	0.314	-1.004	0.244	0.230

Note: *B*-Regression beta coefficient test, Independent variable: Gut health, *CI*-confidence interval, P value significant at *p*<0.05, *WAZ*- Weight- for- Age, *HAZ*-Height-for-Age, *WHZ*- Weight- for- Height Z-Scores

4.6.2 Socio-demographics, dietary, morbidity characteristics and gut health

A linear regression was done to determine the association between some sociodemographic, dietary, morbidity characteristics, and gut health indicators (LRR and 13CSBT). Recent incidences of diarrhea showed significant association with LRR among children (Beta=0.352, P=0.034, 95% Cl-0.023-0.550) **(Table 4.14).**

Table 4.14: **some sociodemographic, dietary, morbidity characteristics, and LR**

Variables	**B**	**t**	**p-value**	**95% CL for B**	
				Lower Bound	**Upper Bound**
Age of the child	-0.08	-0.43	0.67	-0.149	0.098
Gender of the child	-0.22	-1.19	0.24	-0.461	0.122
Mother's age	0.24	1.13	0.27	-0.014	0.049
Marital status	-0.14	-0.68	0.50	-0.541	0.270
Source of drinking water	-0.20	-1.04	0.30	-0.504	0.163
Handwashing with soap	-0.27	-0.74	0.47	-0.877	0.411
Hand washing station	0.06	0.19	0.85	-0.492	0.592
Type of toilet	0.26	1.10	0.28	-0.256	0.849
No. people sleeping, house	-0.15	-0.67	0.51	-0.161	0.081
Respondent education	0.14	0.60	0.55	-0.094	0.172
Monthly income	0.01	0.01	0.99	-0.248	0.250
No. times Breastfed	-0.04	-0.19	0.85	-0.101	0.084
Dietary diversity score	-0.32	-0.50	0.62	-0.411	0.248
Food security status	-0.07	-0.22	0.83	-0.591	0.476
No. times child fed day	0.18	0.77	0.45	-0.053	0.116
Recent diarrhea case	0.35	2.22	**0.03***	0.023	0.550
Worms child's stool	0.05	0.24	0.81	-.332	0.422
Child deworming	-0.01	-0.04	0.97	-0.410	0.393

Note. Dependent variable; LRR, *B*-standardized regression coefficient, *P values significant at $p<0.05$

4.6.4 Some socio-demographics, dietary characteristics and 13CSBT

Linear regression analysis between socio-demographic and dietary characteristics with 13CSBT outcome indicated that, use of soap in hand washing and having a hand washing station in the household indicated significant association with 13CSBT CPDR at 90 minutes among the children at (B=0.687, P=0.044, 95% Cl-0.122-8.162) and (B= -0.628, P=0.042, 95% Cl- -6.902- -0.138) respectively (Table 4.15)

Table 4.15: **Some demographic, dietary, morbidity characteristics and 13CSBT**

Variables	**B**	**t**	**p-value**	**95% CL for B**	
				Lower bound	**Upper bound**
Age of the child	-0.12	-0.67	0.511	-1.021	.519
Gender of the child	-0.10	-0.59	0.560	-2.343	1.292
Mother's age	0.07	0.36	0.723	-0.163	0.232
Marital status	-0.04	-0.22	0.829	-2.800	2.260
Source of water Class	-0.06	-0.33	0.742	-2.418	1.740
Use of soap in handwashing	0.69	2.10	**0.044***	0.122	8.162
Hand washing station	-0.63	-2.12	**0.042***	-6.902	-0.138
Type of toilet	-0.25	-1.19	0.243	-5.463	1.434
No. people sleeping, house	-0.02	-0.08	0.936	-0.785	0.725
Mother level of education	0.038	0.18	0.862	-0.757	0.899
Average monthly income	-0.267	-1.34	0.190	-2.577	0.534
No. times child breastfeed	0.075	0.41	0.688	-0.463	0.693
Minimum dietary Diversity	-0.465	-0.75	0.460	-6.308	2.922
Food security status	-0.373	-1.26	0.218	-5.382	1.279
No. times child fed in 24hrs	-0.047	-0.22	0.830	-0.582	0.470
Recent child diarrhea case	0.019	0.14	0.893	-1.536	1.754
Worms in child's stool	-0.048	-0.28	0.782	-2.676	2.033
Child treated for worms	-0.544	-2.51	**0.017***	-5.006	-0.519
Regular child deworming	0.104	0.44	0.662	-1.963	3.048

Note. Dependent variable: 13CSBT, *cPDR90*- cumulative Percent Dose Recovery at 90 minutes after dosing, *B*-standardized regression coefficient, * significant at $p<0.05$

CHAPTER FIVE: DISCUSSION

5.1 Introduction

This section presents an in depth discussion of the study findings as per each objectives. The discussion provides a comparison of the findings of the current study with the findings of similar studies. The researcher provides a critique to the findings of other studies and gauge their findings.

5.2 Water, sanitation and hygiene situation among children aged 12-15 months, Siaya County, Kenya

The WHO WASH Strategy 2018-2025 underscores the critical role of safe drinking water, basic sanitation, and good hygiene practices for children's survival. Unsafe drinking water and poor sanitation practices expose children to illnesses such as EED, diarrhoea, and Neglected Tropical Diseases (NTDs), including trachoma, soil-transmitted helminths, and schistosomiasis (WHO/UNICEF, 2021).

Current study found that more than half of the households accessed safe water for drinking, such as taped water and protected wells, a rate slightly above the national average of 68%. Access to piped water in homes is essential for ensuring a constant supply of safe drinking water. However, this study found that very few households had piped water in their dwellings. A similar trend was observed in an Indian study, where only 15.6% of households had access to safe drinking water within their homes (Giri *et al*, 2022). This reflects the need for improved infrastructure to enhance access to clean water, especially in resource-limited areas like Siaya County. However, nearly a quarter of households continued to rely on unimproved water sources, which are unsafe, a proportion lower than the national figure, where 44% of the population lacks access to safe water

(KDHS, 2022). Additionally, nearly a third of households reported relying on surface water sources, such as dams and rivers, mirroring the national situation where 9.9 million people depend on such water sources (UNICEF, 2022).

Water sanitation entails access to clean water that is not exposed to human waste, dirt or microbes (WHO, 2023). The sources of drinking water are either protected which is safe for human use with little or no incidences of infections or unprotected that puts the users at risk of intestinal infections and diarrhea (Gizaw *et al*., 2020). The source of water for household activities such as cooking and washing also determines the level of sanitation of the households and exposure to dirt and microbes. Unprotected water source is often contaminated with human and animal waste, exposing children to harmful microbes that can lead to illnesses such as diarrhea. Chronic exposure to these contaminants can contribute to EED, a condition linked to impaired nutrient absorption and growth, which is associated with continuous cycle of malnutrition and increased hospitalization the Country stunting in children (Mshida *et al*., 2018; Giri *et al* ,2022).

UNICEF (2020) reports that only 29% of Kenyans have access to basic sanitation, and just 25% have handwashing facilities with soap and water at home. The current study reflected similar trends, finding low adoption of handwashing practices in resource-constraint rural areas of Siaya County, where some households had limited access to improved water sources. This depicts the ongoing challenges related to WASH in underserved communities. A similar trend of low uptake of sanitation facilities was noted, in Kajiado County, Kenya, with only 9% of households' latrines having a handwashing station (Okumu *et al*., 2022). In contrast, a study in Nepal reported a higher uptake of sanitation practices, such as the use of soap during hand washing at 76%.

(Shrestha *et al.*, 2020). Hand washing has been reported to reduce cases of diarrhea by 30% and sanitation interventions lower the risk of diarrhea morbidity by 25%, with evidence of further reduction by 45% when sanitation coverage of above 75% is attained among children that later results in improved gut health and nutritional status (Wolf *et al*., 2018).

Improper disposal of human excreta is linked to transmission of diseases such as cholera, amoebic dysentery and damaged gut barrier. It is linked to 432000 diarrheal deaths, and increased risk of EED among children, that affect optimal linear growth and development (UNICEF, 2020; Giri et al, 2022). From the study, most of the respondents reported to have pit latrines, Pit latrines without slab have been reported to be most common in Kenya (Njuguna, 2019). Similarly, results were reported by Shrestha *et al*., (2020) in Nepal with 93.76% having pit latrines and 48.7% of them being unhygienic and 6.3% having no toilet at all thus could be practicing open defecation and exposing children to human excreta. Despite the study, not reporting lack of toilet in Siaya there is still some 16% of Kenyan households with no toilets and about 5 million practicing open defecation exposing people to human excreta and microbes (KDHS, 2022).

In Kajiado County Kenya, only 29.7% of the households reported to have pit latrines with only 17.1% of them being improved (Okumu *et al.*, 2022). As reported by Nataro *et al*., (2016) such pit latrines are often shared which further exposes children to fecal bacteria ingestion, excreta contaminants and frequent infections. Long-term exposure to these conditions contributes to diarrheal diseases. EED and poor nutrition status among children.

Research shows that having access to an improved sanitation facilities is estimated to reduce a child's risk of diarrhea by 22% to 36%, and may also have additional impacts on the incidence of infections, EED, wasting, and stunting (Sinharoy *et al*., 2021)

Designating a separate room for food preparation, cleaning, and storage can significantly reduce the risk of cross-contamination within households. This separation minimizes the transfer of pathogens from other household areas, as cooking and food handling spaces can be maintained with stricter hygiene standards. Studies indicate that controlling the environment where food and water are stored ensures safer handling and reduces the likelihood of foodborne illnesses associated with contaminated surfaces and water sources (Merid *et al*., 2023; Wandera *et al*., 2022). However, the study reported that more than half of the respondents lacked a separate room as a kitchen. Overall, the findings shows a very low uptake of water, hygiene and sanitation measures and risks children to microbe's exposure leading to illnesses. Thus as a country we are still way far from meeting the sustainable development goal number 6 on sustainable water and sanitation facilities and 100% open defecation free by 2030.

Inadequate WASH practices are associated with diarrhea-related infections, which in turn are linked to around 50% of cases of undernutrition among children under five globally (UNICEF, 2020). Addressing deficiencies in WASH practices is therefore key for optimal child health outcomes. Enhancing these practices can substantially reduce the incidence of waterborne conditions and associated undernutrition, supporting better growth, immunity, and cognitive development. Evidence suggests that improved WASH infrastructure, combined with community education on hygiene, can lead to marked reductions in diarrhea and EED, contributing to overall healthier environments for young children in resource-limited setting (Prüss-Ustün et al., 2019)

5.3 Gut health of children 12-15 months in Siaya County Kenya

Gut health is important for children under five years old, as it supports digestion, nutrient absorption, and immune development (Gizaw *et al.*, 2020; Ma *et al*., 2024). EED greatly disrupts gut health, especially in low- and middle-income countries where children are continually exposed to environmental microbes and infections due to poor WASH practices (DeBoer & Guerrant, 2019; Lin *et al*., 2020). EED results in nutrient malabsorption, poor growth, and more elevated infection risks (Tickell *et al*., 2019). It impairs the gut structure, weakening cellular tight junctions and creating a permeable barrier that allows bacterial translocation, leading to immune activation and systemic inflammation with long-term health and nutrition impacts (Faubion *et al*., 2016; Tickell *et al*., 2019).

Researchers have linked EED with a more severe impairment of children's gut health compared to other intestinal conditions like diarrhea, celiac disease, and parasite infestation (Koyuncu *et al.*, 2020; Modern *et al*., 2022; Wandera *et al*., 2022). Findings from a study by Gough *et al*., (2020), that used WASH interventions showed no consistent changes in intestinal biomarkers, indicating that WASH alone may not effectively prevent EED. Consequently, EED remains a significant challenge, adversely affecting children's health and nutrition. However, effective diagnostic tests and interventions are still limited, as the elusive nature of EED complicates efforts to improve gut health in children under five (Shivakumar *et al.*, 2024).

The current study showed a high prevalence of EED among children 12-15 months in Siaya County, Kenya compared to an Ethiopian study (50%) among rural children aged 12-16 months (Chen *et al.*, 2021). A study in Peru among children reported similar prevalence (Faubion *et al*., 2016). However, an EED study in Kilifi using lactulose and mannitol sugars showed a significantly higher prevalence (67%) of EED compared to Siaya County (Crane, 2019). The results could be informed by the predominantly resource limited rural setting, high dependency on surface water as source of drinking water and the high prevalence of unimproved sanitation facilities in the area. These factors lead to chronic exposure of children to microbes and infections resulting into EED. Consequently, the gut is impaired contributing to the cycle of undernutrition among children in Siaya County, Kenya.

A review study reported that Zambia had the highest prevalence of EED at 82%, while the USA showed the lowest prevalence at 5 % (Faubion *et al*., 2016). The differences in the prevalence of EED reflects variability in WASH conditions, levels of exposure to contaminants, access to health care and poverty levels across various countries. The brush border effect in the gut can make EED appear transient at times, leading to an observed lower prevalence, as damage or inflammation in the brush border (microvilli), can temporarily mask symptoms and reduce the visibility of EED during assessments (Shivakumar *et al*,.2024; Tickel *et al*,.2022).

However, the apparent reduction is more likely attributable to methodological differences in measuring and reporting EED prevalence rather than an actual decrease in incidence (Shivakumar *et al*, .2024). These methodological variations contribute to inconsistencies in reported EED prevalence rates. For instance, studies in Malawi have reported very high prevalence rates of 80.7%, 83%, and 88% respectively (Ordiz *et al*., 2016; Semba *et al*., 2017; Benzoni *et al*., 2015). A study in rural Gambia reported the highest prevalence of EED (95%) among children 1 to 9 years old in the study, with only 5% reporting normal lactulose mannitol ratio, indicating an extreme burden of gut impairment among children (van der Merwe *et al*, 2013). This could inform the unique results obtained in the current study. However, Tanzania showed very low prevalence of EED compared to other African countries at 9% (Modern *et al*., 2022; Ordiz *et al*., 2016), this observation could indicate improved WASH among the children, methodological challenges or the transient nature of EED at the point of assessment.

In the current study, a substantial difference was noted between children who tested positive for EED and those who did not, as shown by their LRR) outcomes. The high mean LRR among EED-positive children implies pronounced abnormalities in both the gut's absorptive function and its barrier integrity, consistent with the findings by Crane *et al*, (2015) and Shivakumar *et al*, (2024). An increased LRR indicates a compromised gut barrier, where increased lactulose permeability points to a breakdown in the tight junctions between cells, while reduced rhamnose absorption reflects decreased mucosal absorptive capacity. These findings were further backed up by minimal changes in the recovery of $^{13}CO2$ in the ^{13}CSBT (cPDR90), measured between baseline and at 90 minutes. The low cPDR90 values indicate damage to the mucosal layer, which is essential for nutrient breakdown and absorption in the gut (Lee *et al*., 2020).

However, despite LRR showing high prevalence of EED among the children, ^{13}CSBT results were not clear indicative of the EED status among the children. This was occasioned by the low $^{13}CO2$ enrichment in breath samples presumably induced by issues during breath collection, sample storage, or transport and differences in test duration (90 minutes compared to 240 minutes), the ^{13}C-SBT outcomes were not directly comparable to those from other studies as noted by Shivakumar *et al*, (2024). The findings corroborates with Schillinger *et al.*, (2022) study that showed a weak relationship between measured enzyme activity in the gut and 13C-SBT parameters, which suggested that the ability to digest and oxidize sucrose, is maintained even when EED prevalence is high.

The scenario was explained by the inadequate enrichment of the ^{13}C sucrose, given the high consumption of C4 plant diets in the study area (Jaika et al,. 2024). The dietary intake of C4 plants before administering the ^{13}C sucrose was a critical challenge in ^{13}CSBT. C4 plants, such as maize, millet, sugar cane, and sorghum, prevalent in the study area, utilize the enzyme phosphoenolpyruvate carboxylase to metabolize carbon into a four-carbon molecule through carbon fixation (Wang *et al.*, 2012). This results in higher retention of the heavy isotope ^{13}C in the tissues of the individuals consuming these plants. In this study, 74% of the children had consumed sorghum, 68% millet, 61% maize, and 62% sugar cane. The higher 13C retention in children consuming C4 plants significantly influenced ^{13}C-SBT outcomes, leading to higher baselines compared to cumulative percent recovery at 90 minutes (cPDR90) (Cui, 2021; Wang *et al.*, 2012). A diet rich in c4 plants was a big challenge requiring further enrichment for better results in ^{13}CSBT as a novel test for EED among children. The findings agree with a study by Shivakumar *et al*, (2024), which indicated that children who had a minimally diverse diet the day before the test exhibited higher cPDR90 values, further highlighting the role of diet in 13CSBT outcomes.

5.4 Nutrition status of children 12-15 months in Siaya County Kenya

The nutrition status of children is a key determinant of their future health and wellbeing. It is highly dependent on the WASH status of the environment in which the children exist and contributes to EED and diarrheal diseases among these children (Wolf *et al*., 2023). More than half of the children in the study had some form of malnutrition including stunting, wasting, and underweight. High malnutrition levels have long-term impacts on individual nutrition status, cognitive development, and economic potential (Modern *et al*, .2022). The study found lower levels of stunting compared to previous EED studies in Ethiopia, where stunting was reported at 41% among children aged 12-16 months, in rural Bangladesh, where 45% of children aged 6-24 months were stunted, and in Tanzania where it was reported at 19.5% (Chen *et al*., 2021; Campbell *et al*., 2018; Modern *et al*., 2022). Stunting is the most preferred measure of undernutrition in children since it reflects a case of accumulated effects of inadequate nutrients intake over time culminating in impaired linear growth (Budge *et al*., 2019; Regassa *et al*., 2023). Research has linked increasing childhood stunting prevalence to poor WASH situations and EED, both of which contribute to chronic nutrient malabsorption by damaging the gut's mucosal lining. This damage limits nutrient uptake, eventually impairing children's linear growth. This is associated with an increased risk of childhood morbidity and death (Tickel *et al*., 2022; Wandera *et al*., 2022).

Nearly half of the children were reported to be wasted in the study, based on the WHO, (2023) classification, this is a very high level of wasting with clinical significance. Similar findings were noted in a retrospective hospital study in Garissa County that reported child wasting at 95% among children aged 6-59 months, reflecting high rates of wasting in the

County (Wambua et al, .2024). However, the prevalence was high compared to the national statistics of 5% and a study in Tanzania that reported wasting at 3.4% (KDHS, 2022; Modern *et al*., 2022). The high wasting prevalence in the current and related studies echoes the emerging trends across countries, where wasting is on the rise among children with little improvement. Global burden of wasting stands at 45 million, with 13.6 million severely wasted. Severe wasting puts children at an 11-fold risk of dying from infectious diseases compared to children who are not wasted. (UNICEF, 2022). Wasting is the life-threatening and most visible form of undernutrition that result from poor nutrient intake, recurrent illnesses, poor WASH, emergencies and the increasing food insecurity (UNICEF,2022). Children are very sensitive to slightest changes in the environment they exist. Severe wasting among children weakens the immunity, increases susceptibility to long-term developmental delays and an increased risk of death (Koyuncu *et al*., 2020; Wandera *et al*., 2023. Early detection and immediate treatment are essential for children with severe wasting to enhance their chances of survival.

The current study reported a considerably high prevalence of underweight compared to the national and Siaya County statistics, at 10% and 7%, respectively (KDHS,2022). This rate was also higher than that of a Tanzanian study, which reported a prevalence of 5.4% (Modern *et al*., 2024). However, a cross-sectional analysis study in Bangladesh found a comparatively higher underweight prevalence (28.7%) compared to the study outcome (Ghosh *et al*, .2021). Underweight in children is frequently utilized as a general indicator of nutritional status; however, it is a composite measure as it can reflect both acute and chronic forms of malnutrition. Chronic malnutrition (stunting), results from long-term nutritional deficiencies and leads to impaired growth and development, while acute malnutrition (wasting), is typically due to recent, severe weight loss from an immediate lack of adequate food or illness. This dual manifestation of malnutrition types in a single indicator complicates the interpretation of underweight, since it may mask the distinct underlying causes (Wambua et al, .2024). Therefore, while underweight provides a general measure of malnutrition, it lacks specificity regarding the nature and duration of nutritional deficiencies. This complexity needs careful consideration in both diagnosis and intervention planning, as targeted responses may be needed to address chronic versus acute malnutrition effectively (UNICEF, 2022).

The management of undernourished children usually prioritizes wasting because it depicts an acute form of malnutrition with an elevated risk of mortality, demanding prompt nutritional intervention (WHO, 2023; Ghosh et al, .2021). Wasting reflects recent, severe weight loss, often due to a sudden decline in food intake or disease. This condition is treated urgently with calorie-dense therapeutic foods and medical care to stabilize the child's health and avert life-threatening outcomes. Nevertheless, wasting repeatedly coexists with underweight and stunting, which are broader indicators of both chronic and acute malnutrition (Wambua et al, .2024). Underweight is a composite measure that can include elements of both wasting and stunting, while stunting is a sign of chronic malnutrition due to prolonged nutritional deficiencies or recurring illness. Children who are both wasted and stunted are particularly vulnerable, as they face compounded threats to their health and development (Ghosh et al, .2021). An adequate management strategy for undernourished children should thus address both the urgent needs associated with wasting and the underlying, long-term nutritional deficiencies related to stunting or underweight status.

However, undernutrition is still a major problem among children with increasing cases of stunting and wasting every day; the cause is complex and requires multidisciplinary approach to overcome it, since individual interventions like dietary and WASH have failed to address the problem (Shivakumar *et al*., 2024). The ever-rising incidences of stunting and wasting among children 6-24 months despite interventions including complementary feeding has been linked to the role of EED and its impact on gut health, limiting gut absorptive capacity and reduced response to nutritional interventions (Skau *et al.*, 2019;Giri *et al*,.2022). Therefore, the exploration of the role of gut health in managing malnutrition could be a probable solution to the high prevalence's stunting, wasting and underweight among children.

5.5 Water, sanitation and hygiene, gut health and nutrition status among children

5.5.1 Water, sanitation and hygiene and gut health among children 12-15 months

Significant challenges persist in achieving SDG 6, which aims to ensure universal access to safe and sustainable water and sanitation for all. WASH-related problems continue to hinder progress toward this goal, despite its high prioritization (UNICEF, 2023) with aim of reducing childhood morbidity. Poor WASH conditions are closely related to compromised gut health in children, as inadequate access to clean water, sanitation, and hygiene practices forms a breeding environment for harmful pathogens (Tickell *et al.*, 2021). When children are exposed to contaminated water and poor hygiene practices, they are at high risk of intestinal infections, which can lead to gut inflammation and disrupt normal functioning. Chronic infections and inflammations are associated with impaired gut health as indicated by high prevalence of EED in the study (Ramlal *et al*., 2023; Ordiz et al 2016).

In the current study, there was evidence of significant negative relationship between source of water for drinking and EED status among children ($p=0.039$). Similar findings were reported in a Tanzanian study, which also showed a significant relationship between water availability and EED, linking it to growth failure ($p = 0.0055$) (Modern *et al*., 2020). These significant causal relationships may be due to the role water in hand washing, drinking and cooking, lack of which may lead to frequent contamination and diarrhea and subsequent growth failure among affected children.

Availability of handwashing stations and use of soap in washing hands was correlated with EED at $r = - 0.232$, $P<0.02$ and $r= -0.218$, $p=0.029$ respectively. However, toilet type and having separate room as kitchen did not show significant relationship with EED ($p>0.05$). This implies that as households continue to use soap and provide handwashing stations cases of EED continues to reduce among children, indicating the positive role of WASH in management of EED. Similarly, in a WASH interventions study implemented in rural Zimbabwe reported that improved pit latrine, hand-washing stations, liquid soap, point of-use water chlorination, and clean play space did not prevent enteric infections that cause EED (McQuade *et al*., 2024). This indicates that there are numerous other issues of concern including insufficient practice of knowledge of WASH by the caregivers. The actual source of water, individual household hygiene practices, and socio-economic status could influence exposure to enteric pathogens leading to EED other than specific aspects of WASH (Sinhroy *et al*., 2021).

5.5.2 Gut health and nutrition status among children 12-15 months

Research highlights a strong connection between gut health and nutritional status among children under five years (Budge *et al*., 2019; Owino *et al*., 2016). Poor WASH conditions expose children to gut pathogens and parasites, leading to chronic conditions such as EED (Lazar *et al*., 2024). EED damages the intestinal lining disrupts the gut microbiome, and impairs nutrient absorption, resulting in chronic inflammation, malabsorption, and nutrient deficiencies that negatively affect growth and development (Modern *et al*., 2022; Owino *et al*., 2021). Despite the known link between EED and stunting, this study did not find a significant relationship between EED and stunting. Similar findings were reported in Bangladesh, where EED was not associated with stunting among children in their second year of life despite a high prevalence of stunting in the area (Campbell *et al*., 2018). However, the findings contradicts with a number of studies that have reported significant associations between EED and stunting (Ghosh *et al*., 2021; Lin *et al*., 2020;; Modern *et al*., 2022; Budge *et al*., 2019). The lack of evidence for a significant impact of EED on linear growth in this study may stem from its cross-sectional design nature, which limits the ability to track changes over time. EED symptoms can be transient, influenced by dietary changes, infection exposure, and seasonality (Lee *et al*., 2020; Shivakumar *et al*., 2024). This variability complicates the accurate assessment of EED's impact and contributes to inconsistent diagnostic findings and prevalence rates (Shivakumar *et al*., 2024).

Additionally, the literature suggests that children in their second year experience a slower vertical growth rate than infancy (Ritchie *et al*., 2009). It implies that when examining the effects of EED on gut health, it is crucial to consider both standard growth patterns and each child's growth history (Regassa et al., 2023). EED may be relatively transient, meaning that children with elevated markers of EED, such as high LRR, at a single time point may not necessarily experience a higher burden of EED over the preceding or subsequent months (Modern *et al*., 2022;).

This transient nature of EED supports the hypothesis that EED is more transient than chronic in young children (Campbell et al., 2018). Consequently, the insignificance of the relationship between gut health and stunting observed in this study could be explained by the variability and transient nature of EED biomarkers during the first two years of life (Brown *et al*., 2016; Modern *et al*., 2022).

The current study also showed a statistically insignificant relationship between wasting and gut health, despite the high prevalence of wasting reported among children ($p>0.05$). The results were contrary to a study in Bangladesh that showed significant effects of EED, with increasing trajectories in wasting among the children 15-18 months (Campbell *et al*., 2018). The difference could be linked methodological differences that limited the ability to link wasting to EED prevalence, though some sometimes lack statistical significance does not rule out scientific significance of the results (Jaika *et al*., 2024).

However, underweight was significantly associated with improved gut health (OR=3.663, 95% Cl: 0.750-3.413; $p<0.05$). This implies that a one unit change in gut health, contributes to 4 fold reduction in underweight prevalence among children. Similar trends reported in Bangladesh (Campbell *et al*., 2018). Further, WAZ showed significant variation ($P=0.024$) among EED positive and EED negative children with similar variations reflected in children weight ($P=0.022$). This finding suggests the impact of EED on body composition among children (Shivakumar *et al*., 2024; Owino *et al*., 2026). A study showed that children with elevated EED biomarker had relatively low weight gain (Campbell et al., 2018). This could be because EED interferes with effective nutrient absorption into the body for utilization and optimal growth and development (Tickell *et al*., 2019; Budge *et al*., 2019).

5.5.2 Some Demographic, dietary and morbidity factors and child nutrition status

Other factors that would contribute poor nutrition status apart from WASH and EED, included food security, dietary diversity, worm infestation, diarrheal diseases, breastfeeding, maternal education, income and household number of people. They have been shown to also influence gut health and nutrition status of children under-five years because they dictate the WASH status and health seeking behaviors of the households (Sahiledengle *et al*., 2021; Crane *et al*., 2022). Children are easily affected by sudden shocks or changes in the environment they exist. The study investigated link between these intervening factors with LRR and 13CSBT; recent diarrhea incidences were significantly associated with high LRR as indicators of EED (p= 0.044) while use of soap in handwashing and handwashing station were significantly related to 13CSBT as an indicator of EED (p=0.042). However, dietary diversity, food security, breastfeeding and sociodemographic characteristics did not showed evidence of association with EED indicators (P<0.05). This indicates that EED is multifaceted and could be associated with many other factors other than just the WASH practices among households in which the children exist.

CHAPTER SIX: CONCLUSION AND RECOMMENDATION

6.1 Conclusion

6.1.1 Introduction

The chapter comprises of the conclusions drawn from the study findings and the discussion. The researcher has also made recommendations for action and for further research in this section.

6.1.2 Water, Sanitation and hygiene among children in Siaya County, Kenya

The study concluded that there were high rates of poor WASH among the children under five years in Siaya County, Kenya. This was characterized by many households with under-five children in rural areas depending on unimproved water sources, such as surface water, due to a lack of clean municipal water supply. The inadequate sanitation facilities, including dependency on pit latrines without flush and majority lacking of handwashing stations, exacerbating issue, reflecting the need for sewerage systems typical of urban areas. The lack of proper sanitation affects children's food safety, feeding practices, and playing environments, increasing the risk of gut illness. Additionally, the absence of separate kitchen spaces for food preparation and storage compromised food hygiene and safety.

6.1.3 Gut health among children 12-15 months in Siaya County

Over half of the children tested positive for EED, thus high prevalence of gut health impairment linked to the poor nutritional status among the children. This was associated with the poor water, sanitation and hygiene that exposed the children to microbes and parasites that contributed to EED and other diarrheal diseases among children in the study area with the population being in deep rural set ups of Siaya County, Kenya.

6.1.4 Nutrition status of children 12 -15 months in Siaya County

High Prevalence's of stunting, wasting and underweight were reported in Siaya County. This high prevalence's of poor nutrition status was associated with the sub-optimal WASH status and the high prevalence of EED in the study area. EED contribute to impaired gut and limited absorptive capacity that reduce nutrients uptake for body growth and development. The chronically high prevalence of wasting and stunting among children 12-15 months existed despite the complementary feeding done to the children among other interventions. Therefore, there is need to focus on the probable role of EED on the increasing prevalence of undernutrition.

6.1.5 Relationship between water, sanitation and hygiene, gut health and nutrition status

There was a significant link between WASH and gut health among children. Additionally, poor gut health was significantly associated with the prevalence of underweight in children, as it impaired nutrient absorption and utilization. However, stunting and wasting were insignificantly related to gut health, indicating that while poor WASH conditions impacted weight, their effect on linear growth and muscle wasting was less direct or mediated by other factors. Improving WASH conditions may help reduce underweight prevalence, but additional measures are needed to address stunting and wasting effectively.

6.2 Recommendations

6.2.1 Recommendation for policy

The national government, development partners and the County government of Siaya should critically rethink the current public health strategies to enhance effectiveness in ensuring community access and adoption of optimal WASH practices while incorporating related factors like socio-demographic and economic factors in rural areas. This would help in reduction of child exposure to enteric pathogens and reduce the rising prevalence of EED among rural children in Kenya.

The National and County governments should reconsider the determination of gut health in management of malnutrition among children under five years, the current guidelines does not provide for gut health which is key in effective management of malnutrition among children

6.2.2 Recommendation for practice

The National and County government Siaya should expand the nutrition screening programs to the rural areas for early detection and referral of malnutrition related cases. They should engage development partners for a coordinated approach to screening and interventions. They can also engage the community health promoters in enhancing effective screening.

6.2.3 Recommendation for further research

Future researchers should conduct a longitudinal study over at least one year, involving a larger sample size and repeated EED assessments, to analyze the impact of WASH on EED and its effect on children's nutritional status, particularly stunting. This approach will capture trends over time and establish causal relationships more effectively than single-point assessments.

Furthermore, researchers intending to use 13CSBT for gut health determination should consider a more highly enriched 13C-sucrose sugar for the test to overcome the effects of C4 plant sources of food in similar study settings to get accurate diagnosis of EED.

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APPENDICES

Appendix I: Parent/Guardian Informed Consent Form

Title of Study: **Gut Health and Nutrition Status among Children with Compromised Water, Sanitation and Hygiene in Siaya County, Kenya.**

Principal Investigator and institutional affiliation: Silvester Ndori Jaika, MMUST

Co-Investigators and institutional affiliation: Dr Konyole S., MMUST

Introduction

I would like to tell you about a study being conducted by the above listed researchers. The purpose of this consent form is to give you the information you will need to help you decide whether or not your child should participate in the study. Feel free to ask any questions about the purpose of the research, what happens if your child participates in the study, the possible risks and benefits, the rights of your child as a volunteer, and anything else about the research or this form that is not clear. When we have answered all your questions to your satisfaction, you may decide if you want your child to be in the study or not. This process is called 'informed consent'. Once you understand and agree for your child to be in the study, I will request you to sign your name on this form. You should understand the general principles which apply to all participants in a medical research: i) Your child decision to participate is entirely voluntary ii). You child may withdraw from the study at any time without necessarily giving a reason for his/her withdrawal iii) Refusal to participate in the research will not affect the services your child is entitled to in this health facility or other facilities.

May I continue? YES / NO

For children below 18 years of age we give information about the study to parents or guardians. We will go over this information with you and you need to give permission in order for your child to participate in this study. We will give you a copy of this form for your records.

What is the purpose of the study?

The researchers listed above are interviewing individuals who have children aged 12-15 months attending Siaya County referral hospital. The purpose of the interview is to find out the influence of Gut integrity and WASH on the nutrition status of the child. Participants in this research study will be asked questions about the socio-demographic characteristics of the child, water, sanitation and hygiene situation, anthropometric assessment mother's education. Participants will also have the choice to undergo test such as Dual sugar test (L:R) and 13C SBT for gut permeability, Stool for intestinal parasites .

There will be approximately 100 participants in this study randomly chosen. We are asking for your consent to consider your child to participate in this study.

What will happen if you decide you want your child to be in this research study?

If you agree for your child to participate in this study, the following things will happen:

A trained interviewer in a private area where you feel comfortable answering questions will interview you. The interview will last approximately 30 minutes. The interview will cover topics such as socio demographic data of both child and mother, water, sanitation and hygiene, anthropometrics, benefits, risks and limits of the study and the procedures involved.

After the interview is finished, your child will undergo several procedures including counselling, breath collection, urine collection (After dual sugar swallow), deuterium solution swallow (for body composition), stool for intestinal parasites. Specimen of your child's urine, breath, blood and stool will also be preserved and stored for up to five years. This samples will be analyzed to determine their body composition, presence of parasites and permeability of the gut. You will be informed about the results.

We will ask for a telephone number where we can contact you if necessary. If you agree to provide your contact information, it will be used only by people working for this study and will never be shared with others. The reasons why we may need to contact you include: provision of test results, follow up, any information that may be important to you.

Are there any risks, harms, discomforts associated with this study?

Medical research has the potential to introduce psychological, social, emotional and physical risks. Effort should always be put in place to minimize the risks. One potential risk of being in the study is loss of privacy. We will keep everything you tell us as confidential as possible. We will use a code number to identify your child in a password-protected computer database and will keep all of our paper records in a locked file cabinet. However, no system of protecting confidentiality can be absolutely secure so it is still possible that someone could find out your child was in this study and could find out information about your child.

Also, answering questions in the interview may be uncomfortable for you. If there are any questions you do not want to answer, you can skip them. You have the right to refuse the interview or any questions asked during the interview.

It may be embarrassing for you to have samples of urine picked, breath and stored from your child. We will do everything we can to ensure that this is done in private. Furthermore, all study staff and interviewers are professionals with special training in these examinations/interviews. Also, the procedures may take long time and rigorous thus may be stressful.

Your child may feel some discomfort when collecting blood samples or during solution swallowing and may have a small bruise or swelling in on the pricked finger. In case of an injury, illness or complications related to this study, contact the study staff right away at the number provided at the end of this document. The study staff will treat your child for minor conditions or refer the child for treatment for conditions that require more extensive care.

Are there any benefits being in this study?

Your child may benefit by receiving free testing and treatment. You may be counseled on importance of WASH on child's growth, good nutrition and general health information among others. We will refer your child to a hospital for care and support if necessary. Also, the information you provide will help us better understand the child's environment at home, the current health of the child and feeding patterns. This information is a major contribution to science and the government in improving the health condition of the citizens by formulating appropriate policies.

Will being in this study cost you anything?

The study will involve spending time with the child at the study site as the researchers continue with their work therefore it will cost the caregiver some considerable time at most one day. The participants will have to incur some transport cost to and from the study site.

Is there reimbursement for participating in this study?

There will not be direct material gain from the study. However, treating will be provided to the child freely during the study period.

What if you have questions in future?

If you have further questions or concerns about your child participating in this study, please call or send a text message to the study staff at the number provided at the bottom of this page.

For more information about your child's rights as a research participant you may contact the Secretary/Chairperson, MMUSTIERC on ierc@mmust.ac.ke . The study staff will pay you back for your charges to these numbers if the call is for study-related communication.

What are your other choices?

Your decision to have your child participate in this research is voluntary. You are free to decline or withdraw participation of your child in the study at any time without injustice or loss of benefits.

Just inform the study staff and the participation of your child in the study will be stopped. You do not have to give reasons for withdrawing your child if you do not wish to do so. Withdrawal of your child from the study will not affect the services your child is otherwise entitled to in this health facility or other health facilities.

For more information contact Silvester Ndori 0712728692 or Prof. Konyole 0773349142 at MMUST from 8am to 5pm.

Consent form (statement of consent)

The person being considered for this study is unable to consent for him/herself because he or she is a minor (a person less than 18 years of age). You are being asked to give your permission to include your child in this study.

Parent/guardian statement

I have read this consent form or had the information read to me. I have had the chance to discuss this research study with a study counselor. I have had my questions answered by him or her in a language that I understand. The risks and benefits have been explained to me. I understand that I will be given a copy of this consent form after signing it. I understand that my participation and that of my child in this study is voluntary and that I may choose to withdraw it any time.

I understand that all efforts will be made to keep information regarding me and my child's personal identity confidential.

By signing this consent form, I have not given up my child's legal rights as a participant in this research study.

I voluntarily agree to my child's participation in this research study:

Yes No

I agree to have my child undergo 13CSBT, Dual sugar: Yes No

I agree to have (urine, breath) preserved for later study: Yes No

I agree to provide contact information for follow-up: Yes No

Parent/Guardian signature /Thumb stamp: _______________ Date ____________________

Parent/Guardian printed name: ____

Researcher's statement

I, the undersigned, have fully explained the relevant details of this research study to the participant named above and believe that the participant has understood and has knowingly given his/her consent.

Printed Name: ____________________________________ Date: _________________________

Signature: ___________________________________

Role in the study: __________________________________ [i.e. study staff who explained informed consent form.]

Witness Printed Name (If witness is necessary) ________________________________

Signature: ______________________________________ Date; _____________________

Appendix II: Structured Questionnaire.

Child ID Code: | | | | | | | |

Enrolment Form Version 1.1			
	Question	**Code**	**Response**
01	Study researcher/Fieldworker ID		
02	Today's date (DD/MMM/YY)		/ /
A. Child information and anthropometry			
01	Date of birth (DD/MMM/YY)		/ /
02	Sex of child	Male=01 Female=02	
03	Birthweight (kg) * (from birth record, if available)		.
04	Current weight (kg) *		.
05	Current length (cm)		.
06	Current head circumference (cm)		.
B. Maternal anthropometry			
01	Mother's date of birth		/ /
02	Is the mother currently pregnant?	Yes = 01 No = 00	
03	Mother's Weight (kg)		.
04	Mother's Height (cm)		.
C. WASH and Socio-economic Information (WAMI) *Please explain to the mother that these questions are standardized questions used around the world, so some questions may be more applicable to them than others.*			
01	Was this child chosen for participation in the study	Low SES= 01 High SES = 02	

	because they come from a low-SES community, or a high SES community?		
02	What is the main source of drinking water for members of your household?	Piped into dwelling = 01 Piped to yard/plot = 02 Public tap/stand pipe= 03 Tube well or borehole = 04 Protected well = 05 Unprotected well = 06 Surface water (river/ dam/ lake/pond/ stream/canal/irrigation canal) = 07 Other = 08	☐☐
02a	If other, describe:		
03	What is the main source of water used by your household for other purposes such as cooking and hand-washing?	Piped into dwelling = 01 Piped to yard/plot = 02 Public tap/stand pipe= 03 Tube well or borehole = 04 Protected well = 05 Unprotected well = 06 Surface water (river/ dam/ lake/pond/ stream/canal/irrigation canal) = 07 Other = 08	☐☐
03a	If other, describe:		
04	What kind of toilet facility do members of your household usually use?	No facility/bush/field or bucket toilet = 01 Pit latrine without flush = 02 Flush to piped sewer system = 03 Flush to septic tank = 04 Flush to pit latrine = 05 Flush to somewhere else = 06 Other = 07	☐☐
04a	If other, describe:		
05	Do you have a separate room which is used as a kitchen?	Yes = 01 No = 00	☐☐
06	Does any member of your household have a bank account?	Yes = 01 No = 00	☐☐

07	Does your household have a mattress?	Yes = 01 No = 00	
08	Does your household have a refrigerator?	Yes = 01 No = 00	
09	Does your household have a television?	Yes = 01 No = 00	
10	Does your household have a table?	Yes = 01 No = 00	
11	Does your household have a chair or bench?	Yes = 01 No = 00	
12	How many rooms are there in your house?	Yes = 01 No = 00	
13	How many people usually sleep in this household?	01-30 (people)	
14	Have you (the mother of the study child) ever attended school? *If no, skip to question 18.*	Yes = 01 No = 00	
15	How many years of schooling have you completed?	00-20	
16	*If younger than 25 years old:* Are you currently attending school or college?	Yes = 01; No = 00	
17	What is the average monthly income for the entire household?		
18	Currency	;S=Kenyan Shilling	
D. Child's dietary diversity			
01	Are you breastfeeding <CHILD>? If NO, then skip to Q.6	Yes = 01 No = 00	
01a	Last night, how many times did you breastfeed <CHILD> from sunset to sunrise?	00-99	
01b	Yesterday, during the day, how many times did you breastfeed <CHILD>?	00-99	

02	Do you give <CHILD> infant formula? If NO, then skip to Q.9	Yes = 01 No = 00	☐☐
02a	Last night, how many times did you feed <CHILD> formula from sunset to sunrise?	00-99	☐☐
02b	Yesterday, during the day, how many times did you feed <CHILD> formula?	00-99	☐☐
03	Do you give <CHILD> other milks, such as tinned, powdered or fresh animal milk? If NO, then skip to Q.12	Yes = 01 No = 00	☐☐
03a	Last night, how many times did you feed <CHILD> animal milks from sunset to sunrise?	00-99	☐☐
03b	Yesterday, during the day, how many times did you feed <CHILD> animal milk?	00-99	☐☐
Yesterday, during the day or last night, did <CHILD> have:			
04	Plain water	Yes = 01 No = 00	☐☐
05	Tea, coffee <local examples>?	Yes = 01 No = 00	☐☐
06	Fruit or vegetable juices?	Yes = 01 No = 00	☐☐
07	Any other liquids, such as sugar water, thin soup or broth, carbonated drinks <local examples>	Yes = 01 No = 00	☐☐
08	Is <CHILD> eating any semi-solid, mashed or solid foods? If NO, go to Q24	Yes = 01 No = 00	☐☐
Thinking about yesterday, during the day and at night, did <CHILD> have any of these foods, even if they were in combination with other foods?			
09	Maize?	Yes = 01 No = 00	☐☐

10	Sorghum?	Yes = 01 No = 00	
11	Millet (any kind)?	Yes = 01 No = 00	
12	Sugar cane or cane-derived sugar	Yes = 01 No = 00	
13	Rice, porridge, bread, noodles or other foods made from grains?. (do not include foods made from maize, sorghum, or millet) Mention <local examples>	Yes = 01 No = 00	
14	White potatoes, white yams, manioc, or other foods made from roots? Mention <local examples>	Yes = 01 No = 00	
15	Carrots, squash, or sweet potatoes that are yellow or orange inside? Mention <local examples>	Yes = 01 No = 00	
16	Any dark green leafy vegetables such asspinach? Mention <local examples>	Yes = 01 No = 00	
17	Foods made with beans, lentils, peas, corn, ground nuts? Mention <local examples>	Yes = 01 No = 00	
18	Ripe mangoes, papayas, or other sweetyellow/orange or red fruit? Mention <local examples>	Yes = 01 No = 00	
19	Any other fruits or vegetables such as banana, apple, oranges, tomatoes, avocado? Mention <local examples> (not including sugar cane)	Yes = 01 No = 00	
20	Liver, kidney, heart or other organ meats?	Yes = 01 No = 00	

	Mention <local examples>		
21	Any meat, such as chicken, beef, lamb, goat, duck (others)? Mention <local examples>	Yes = 01 No = 00	☐☐
22	Eggs? Mention <local examples>	Yes = 01 No = 00	☐☐
23	Fresh or dried fish or shellfish? Mention <local examples>	Yes = 01 No = 00	☐☐
24	Cheese, yogurt or other dairy products? Mention <local examples>	Yes = 01 No = 00	☐☐
25	Any sugary foods such as pastries, cakes or biscuits? Mention <local examples>	Yes = 01 No = 00	☐☐
26	Any commercially available foods for infants or young children? Mention <local examples>	Yes = 01 No = 00	☐☐
27	Yesterday, counting meals and snacks, how many times did you feed <CHILD>?	00-99	☐☐
28	How would you describe your child's appetite? Would you say it is:	01=poor, 02=fair, 03=good, 04=very good	☐☐
E. Food Security (HFIAS)			
01	In the past four weeks, did you worry that your household would not have enough food?	No = 00 Rarely = 01 Sometimes = 02 Often = 03	☐☐
02	In the past four weeks, were you or any household member not able to eat the kinds of foods you preferred because of a lack of resources?	No = 00 Rarely = 01 Sometimes = 02 Often = 03	☐☐

03	In the past four weeks, did you or any household member have to eat a limited variety of foods due to a lack of resources?	No = 00 Rarely = 01 Sometimes = 02 Often = 03	
04	In the past four weeks, did you or any household member have to eat some foods that you really did not want to eat because of a lack of resources to obtain other types of food?	No = 00 Rarely = 01 Sometimes = 02 Often = 03	
05	In the past four weeks, did you or any household member have to eat a smaller meal than you felt you needed because there was not enough food?	No = 00 Rarely = 01 Sometimes = 02 Often = 03	
06	In the past four weeks, did you or any other household member have to eat fewer meals in a day because there was not enough food?	No = 00 Rarely = 01 Sometimes = 02 Often = 03	
07	In the past four weeks, was there ever no food to eat of any kind in your household because of lack of resources to get food?	No = 00 Rarely = 01 Sometimes = 02 Often = 03	
08	In the past four weeks, did you or any household member go to sleep at night hungry because there was not enough food?	No = 00 Rarely = 01 Sometimes = 02 Often = 03	
09	In the past four weeks, did you or any household member go a whole day and night without eating	No = 00 Rarely = 01 Sometimes = 02 Often = 03	

	anything because there was not enough food?		
F. Child Morbidity			
01	Does the child have diarrhea today?	Yes = 01 No = 00 Doesn't know = 88	☐☐
02	Over the past 1 week (including today), has your child had diarrhea?	Yes = 01 No = 00 Doesn't know = 88	☐☐
03	If yes to 02, for how many days?	01-07	☐☐
04	Over the past 4 weeks (including today), has your child had diarrhea?	Yes = 01 No = 00 Doesn't know = 88	☐☐
05	If yes to 04, how many separate episodes?	01-20 Doesn't know = 88	☐☐
06	How many days per episode? *Note: Episodes must be separated by at least 2 days without diarrhea*	01-20 Doesn't know = 88	a. First episode ☐☐ days b. Second episode ☐☐ days c. Third episode ☐☐ days
07	In how many episodes was blood/pus/mucus seen? *(The total number of episodes*	01-20 Doesn't know = 88	☐☐
CHRONIC DIARRHEA (Change in consistency of stools with passing of loose or watery stools lasting for MORE THAN 14 days)			
08	Over the past 4 weeks (including today), has your child had diarrhea for MORE THAN 14 days?	Yes = 01 No = 00 Doesn't know = 88	☐☐
09	Were there any hospitalizations in the last 4 weeks? *If no, skip to Q 2.12. If yes, record each hospitalization separately*	Yes = 01 No = 00 Doesn't know = 88	☐☐
09a	Date of first admission		☐☐ / ☐☐ / ☐☐
09b	Diagnosis:		

09c	Date of second admission		__ __ / __ __ / __ __
09d	Diagnosis:		
History of worm infestation			
10	Have you observed worms in your child's stools	Yes = 01 No = 00 Doesn't know = 88	__ __
11	Has your child been treated for worm infestations in the last 6 months?	Yes = 01 No = 00 Doesn't know = 88	__ __
12	If yes, what is the medication taken? (ask for empty syrup bottle/ prescription for medicine details)		
13	Is your child on regular deworming medication?	Yes = 01 No = 00 Doesn't know = 88	

Morbidities	Does child have symptom today?	If yes, how many days in past week including today? (1-7)	Has child had symptom in past 1 month? c	If yes, how many episodes in the past month? (1-28) d	Has child had symptom in past 3 months, if yes how many episodes?

Appendix III: 13C-Sucrose Breath Test: Non-Invasive Assessment of Small Intestinal Function in Children

1. INTRODUCTION

The following Standard Operating Procedure (SOP) details the procedures to follow when measuring the small intestinal sucrase activity of humans *in vivo*.

2. PHYSIOLOGICAL PRINCIPLE

The use of highly enriched ^{13}C-sucrose as a breath test substrate enables a simple and sensitive, non-invasive assessment of small intestinal sucrase activity. It is also a faster and less labour-intensive method of assessing sucrase activity than the *in vitro* sucrase assay. When ^{13}C-sucrose is digested by small intestinal sucrase, its ^{13}C-monosaccharide products, glucose and fructose, are absorbed across the gastrointestinal wall into the bloodstream. From here they travel to the liver, where they are converted to $^{13}CO_2$, which is subsequently exhaled in the breath. Breath $^{13}CO_2$ levels following the ingestion of ^{13}C-sucrose represent the activity of sucrase, or the digestive and absorptive capacity of the small intestine. A diminished level of small intestinal sucrase, for example due to damage or disease, would result in lower levels of breath $^{13}CO_2$ following ^{13}C-sucrose ingestion.

3. MATERIALS & METHODS

Equipment

Breathe Test

- Labco Exetainers -evacuated tubes Labco
- Cannula apparatus
  - 3 way tap
  - PVC tubing (1mm ID, 2mm OD) – Portex
  - PVC tubing (ID 3.97mm, OD 5.56mm, wall 0.79mm) – Tygon
  - Single hole punch
  - Blunt syringe needle
  - 20 - 30ml syringe
- Face-mask apparatus
  - Face mask and collection bag
- $^{13}C_{12}$-sucrose
- Water (potable or bottled water)

Methods

Preparatory work

- Prepare1 g lactulose and 0.2 g rhamnose and dissolve in 15 ml water (~300 mOsmol/L). Using a clean, sterile pipette, transfer into the lactulose/rhamnose solution the appropriate volume of stock $^{13}C_{12}$ sucrose solution
- Label 12 x 12 ml exetainer tubes in an appropriate rack coded with study code, site code, participant code, timepoint, and replicate identifier (e.g. SBT-ZAM1-P1-T60-A).
- Label 12 x 12 ml replicates tubes for storage and code them similarly except for replicate identifier which is labelled B (e.g. SBT-ZAM1-P1-T60-B).

Commencing the test

1. Allow child to settle in the unit and adjust to study surrounding. This may include allowing the child to play with the breath sampling equipment.
2. Fast participants for 1 hour prior to sucrose breath test.
3. The first (baseline) sample is collected immediately **prior to** administration of the ^{13}C-sucrose. Collect baseline breath samples by either:
4. Option 1: Cannula collection technique – place cannula just underneath the nostril and collected exhaled breath using retraction of syringe
5. Option 2: Face-mask collection technique – place face-mask over child's mouth and nose and collect breath until the bag is filled.
6. Transfer exhaled breath to exetainer either by positive displacement (syringe) or using evacuated exetainer (bag).
7. Administer and L/R/^{13}C sucrose solution and wash tube with 5ml and give to child.
8. Continue to collect breath samples as outlined in the protocol.
9. For urine collection empty the urine void from the urine bags / plastic diaper with sterile cotton pad from each time point into a pre-weighed 50 ml falcon tube (1 or 2 depending on the volume of urine voided) with an ice sleeve, once the sample collection is complete weigh the falcon tube to get the total volume of urine. Samples should be stored at -20°C.

10. Breath samples should be stored at room temperature in a box (e.g. in a cupboard). Samples are stable for at least six months.

Criteria for test failure

- Spitting or vomiting the test solution
- Child is withdrawn from the study before 90 minutes
- Failure to collect 6 or more, or 2 consecutive breath samples prior to 90 minutes
- Failure of urine collection
- Breach of exclusion criteria identified retrospectively

Appendix IV: Urine and Breath Collection Form

Child ID Code: | | | | | | | |

Urine and Breath Collection Form Version 1.2 (1 hour post-prandial)			
	Question	**Code**	**Response**
01	Study researcher/Nurse/Fieldworker ID		
02	Today's date (DD/MMM/YY)		/ /
Screening questions			
03	Diarrhea in past month	Yes = 01 No = 00	
04	Antibiotics in past month	Yes = 01 No = 00	
05	Anti-inflammatories in past month *(ibuprofen, naproxen, aspirin, methenozol)* *(paracetamol is OK)*	Yes = 01 No = 00	
If any of these questions (03, 04, 05) was yes, please re-schedule the test one month from the date of the diarrhea or antibiotic/NSAID use			
06	Before starting the test, when was the last time the child ate (either breastmilk or solid foods)	Time (24 Hr Scale; HH:MM)	:
If less than one hour ago, please wait until the child has fasted for one full hour .			

The child can have a breakfast on the morning of the test as long as it was at least one hour ago.			
07	How many loose stools were passed during the breath/urine collection?	Range (00-15, NA)	☐☐
08	Did any breastfeeding occur in the first 90 minutes of the test? (if the mother needed to comfort the child)	Yes = 01 No = 00	☐☐
09	Did the child consume any non-breastmilk liquids or foods during the first 90 minutes of the test? (excluding water, which should be encouraged throughout the test)	Yes = 01 No = 00	☐☐

BASELINE BREATH COLLECTION		
Breath sample tube number	**Time of completion**	**Comments (note crying, difficult with sample collection, etc.)**
Baseline		

BASELINE URINE COLLECTION (LR)			
Urinary collection Time*	**Collection Volume**	**Time of Collection**	**Comments (please note any spillage)**
Baseline			

<u>DO NOT</u> ADMINISTER SUGAR SOLUTION UNTIL THE CHILD HAS FULLY VOIDED THEIR BLADDER		
Total volume (mL) of sugar solution consumed		☐☐ . ☐
Vial number of sugar collection		☐☐
Time the sugar solution was first consumed:		☐☐ : ☐☐
How many minutes did it take the child to consume the dose in full?	01-20 minutes	☐☐
If the child spits out, vomits, or fails to swallow all the sugar solution, the test cannot be completed. Please stop and re-schedule the test for another day.		
Start collection: Encourage the child to drink water throughout the test.		

Breath sample tube number	**Exact collection time *(use a stopwatch)***	**Comments (please note if the child was crying)**
15 minutes		
30 minutes		
45 minutes		
60 minutes		
75 minutes		
90 minutes		

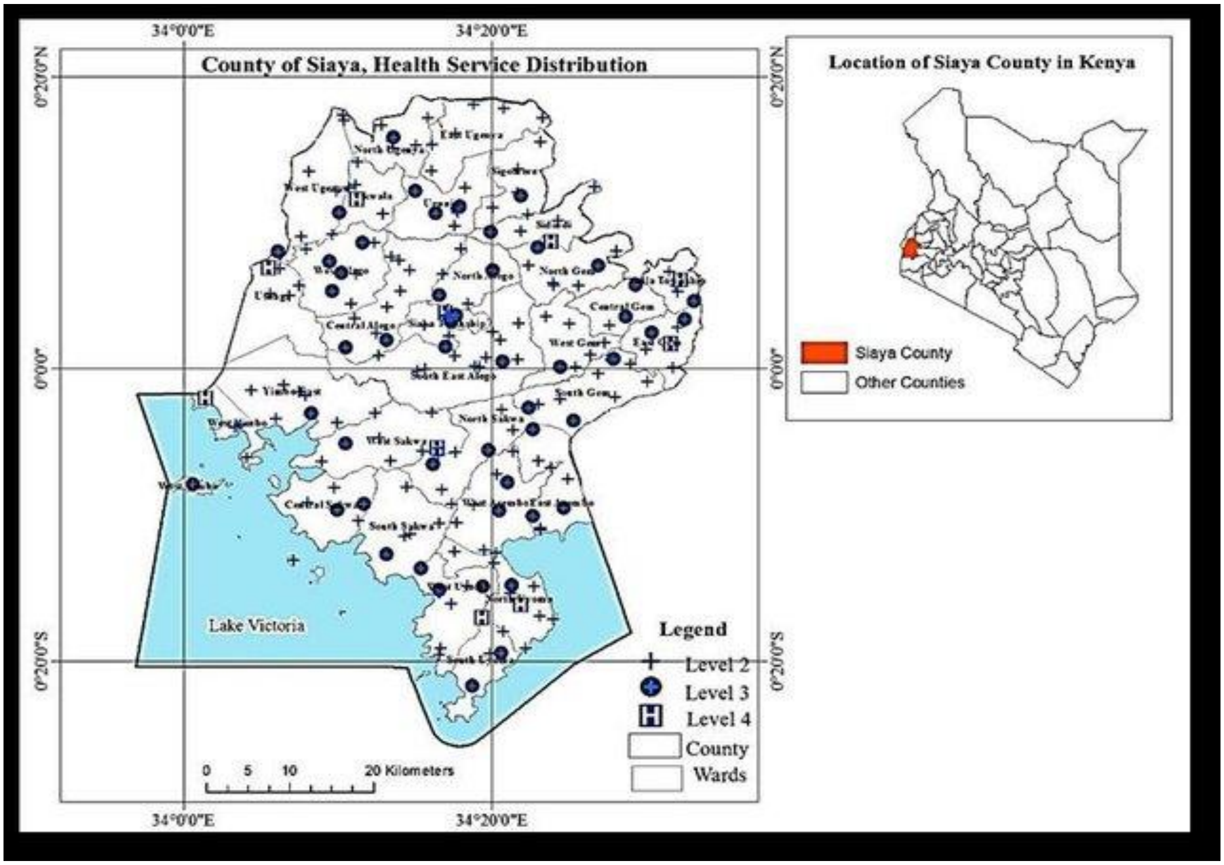
Urinary collection Time	**Collection Volume (mL)**	**Exact collection time *(using a stopwatch)***	**Comments (please note any spillage)**
Do not collect urine for the first 30 minutes after the child consumes the sugar solution:			
30 to 90 minutes			

Appendix V: Summary of Ethical Considerations

S/N	**Principle**	**Ethical concern**	**How it will be addressed**
1	Voluntarism	Participant informed consent.	Participants (care givers) was clearly informed of the purpose of the study and the fact that participation is voluntary and that participation can be terminated at any time the respondent desires. Upon confirmation that the intended participant has understood the terms of their participation in the study, he/ she will be requested to give verbal consent which was noted. Where need be an assent form was administered for minors. All information about the number of tests to be done on the samples, benefits, risks and any relevant concern will be addressed before consent /assent is taken
2	Privacy and confidentiality	Questionnaires	The study design requires that a questionnaire be filled documenting the social economic status, Water and sanitation status, anthropometric data and bio-data for the children and care givers. Codes/serial numbers was used to represent the study participants to ensure confidentiality. The data from the research was used solely for the study and will not be shared. The interview was done in an enclosed area.
3	Confidentiality	Data Security	All data, including audio recordings, was labelled with serial numbers for reasons of anonymity. Names and any personal information collected was kept confidential. The researchers will not reveal the identity of anyone interviewed, beyond groupings by position (ex. Nurses, CHVs). No names will be mentioned in any reports. During the data collection processes, all data will be transferred to the Research Coordinator each evening to be kept on 2 separate external hard drives. The hard drives will be kept in a locked room or on the person of the Research Coordinator at all times. Once the formative research data

			collection, analysis and reporting is complete, all data and questionnaires or audio files will be transferred to the university, to be kept on a secure server for 6 years. No samples will be taken away without consent.
4	Justice/respecter rights	Interviewer/respondent gender issues	Male interviewers were have been trained on the need to ensure that interviews with female respondents are conducted in a neutral space and where necessary the spouse/ partner is informed of the purpose of the interview. Similarly, female interviewees were been advised not to interview male respondents in a secluded setup. To address power issues between male/female or female/ male interviews, the interviewers will be trained on the need to be respectful, firm and culturally sensitive.
5	Beneficence	Feedback to respondents	Part of the workshop in the first learning cycle will be to feedback on the findings from the formative research. Relevant Siaya County-stakeholders, including study communities will be invited to participate findings from the formative research. .
6	Confidentiality/ justice	Reporting of rights violations	An anonymous feedback mechanism was established so that survey /study participants had a method to report any perceived violations of their rights in sample collection and interviews.
7	Voluntarism/informed consent	Taking of Photographs	Photographs were taken following informed consent of the subjects and only used for purposes of the study.
8	Beneficence	Token for participation in the study	Due to the fact that sample collection takes up a considerable portion of the participant's day, a token /food may be provided to participants.

Appendix VI: Map of Siaya County: Health Facilities



Appendix VII: MMUST IERC Approval

MASINDE MULIRO UNIVERSITY OF SCIENCE AND TECHNOLOGY

Tel: 056-31375
Fax: 056-30153
E-mail: ierc@mmust.ac.ke
Website: www.mmust.ac.ke

P. O. Box 190-50100
Kakamega,
KENYA

Institutional Ethics and Review Committee (IERC)

REF: MMU/COR: 403012 Vol 5 (01)

Date: 16th August, 2021

To: Silvester Ndori Jaika

Dear Sir,

RE: GUT HEALTH AND NUTRITION STATUS AMONG CHILDREN WITH COMPROMISED WATER, SANITATION AND HYGIENE.

This is to inform you that ***Masinde Muliro University of Science and Technology Institutional Ethics and Review Committee (MMUST-IERC)*** has reviewed and approved renewal of your above research proposal. Your application approval number is **MMUST/IERC/012/2021.**The approval period is ***16th August, 2021 - 16th August, 2022.***

This approval is subject to compliance with the following requirements;

i. Only approved documents including informed consents, study instruments, MTA will be used.
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by ***MMUST-IERC.***
iii. Death and life-threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to ***MMUST-IERC*** within 72 hours of notification
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to ***MMUST-IERC*** within 72 hours
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to ***MMUST-IERC***.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://research-portal.nacosti.go.ke and also obtain other clearances needed.

Yours Sincerely,

Dr. Gordon Nguka (PhD)
Chairman, Institutional Ethics Review Committee

Copy to:
- The Secretary, National Bio-Ethics Committee
- Vice Chancellor
- DVC (PR&I)

Appendix VIII: NACOSTI Research Permit

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **240546**

Date of Issue: **24/September/2021**

RESEARCH LICENSE

This is to Certify that Mr.. silvester Ndori Jaika of Masinde Muliro University of Science and Technology, has been licensed to conduct research in Siaya on the topic: GUT HEALTH AND NUTRITION STATUS AMONG CHILDREN WITH COMPROMISED WATER, SANITATION AND HYGIENE IN SIAYA COUNTY, KENYA for the period ending : 24/September/2022.

License No: **NACOSTI/P/21/13254**

240546

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

Appendix IX: Siaya County Department of Health Approval

COUNTY GOVERNMENT OF SIAYA

DEPARTMENT OF HEALTH AND SANITATION

E-mail: siayachd@gmail.com
ADJACENT TO JCC CHURCH
PHONE:
SIAYA TOWN

COUNTY HEALTH HEADQUARTERS
SIAYA COUNTY
P O BOX 597
SIAYA

Our Ref: CGS/CHD/RESEARCH/VOL. III (61) **11TH MARCH, 2020**

The Medical Superintendent
Siaya County Referral Hospital

RE: CLEARANCE TO CONDUCT A STUDY ON GUT HEALTH AND NUTRITION STATUS AMONG CHILDREN WITH COMPROMISED WATER, SANITATION AND HYGIENE IN SIAYA COUNTY

Silvester Ndori Jaika Principal Investigator for the above referenced study, has received authorization from Masinde Muliro University of Science and Technology (Reference Number MMU/COR:403012 VOL. 2 (20) to conduct the research in our County.

The main objectives of the study are:

1. To assess the water, sanitation and hygiene situation among children aged 12-15 months in Siaya County
2. To determine the gut health of children 12-15 months in Siaya County, using the 13 carbon sucrose breath test (13CSBT)
3. To assess the nutrition status of children aged 12-15 months in Siaya County

This is to notify you that the research has been authorized by the office of the undersigned.

Kindly accord the team necessary support.

Yours,

COUNTY DIRECTOR OF HEALTH
SIAYA COUNTY
11 MAR 2020
P.O. Box 597-40600,
SIAYA.

Kennedy Oruenjo, MCHD,MPH,BCHD,Dip (EHS)
County Director of Public Health
SIAYA

CC: 1. CECM – Health
2. COH
3. CDMS

Appendix X: Ministry of Health Approval

MINISTRY OF HEALTH
OFFICE OF THE DIRECTOR GENERAL

Telephone: Nairobi 254-020-2717077
Email: dghealth2019@gmail.com

When replying please quote:

Afya House
Cathedral Road
P.O. Box 30016-00100
NAIROBI

REF: MOH/ADM/1/1/82(154)

7th January, 2022

Mr. Silvester Ndori Jaika,
Co-Principal Investigator
Masinde Muliro University of Science and Technology
P.O. BOX 5010-190 Kakamega Webuye Rd,
Kakamega, Kenya
Email: silvesterndori39@gmail.com/konyole2000@yahoo.com

RE: EXPORT PERMIT TO SHIP BIOLOGICAL SAMPLES TO AUSTRALIA, ZAMBIA, AND USA

We refer to your letter dated 10th November, 2021 requesting for a permit to export biological samples to Flinders Medical Centre, Australia, Tropical Diseases Research Centre, Zambia and University of Virginia, USA for a study titled **"Gut health and nutrition status among children with compromised water, sanitation and hygiene in Siaya County, Kenya".** As indicated in the table below:

Sample	Specimen Type	No. of Aliquots	Total	Environmental Enteric Dysfunction (EED) Analysis
Breath Sample (Novel Test)	Human air	4*100	400	Analysis for gut health
Urine Sample (Standard test)	Human Excrete	2*100	200	Lactulose: Rhamnose ratio for gut health
Saliva Sample	Human saliva	3*255	765	Assess saliva composition in relation to gut health

The purpose of this letter is to inform you that this office has **No Objection** to the export of the samples to:

Dr. Roger Yazbek,
Flinders Medical Centre

ISO 9001:2015 certified

South Road
Department of Surgery, level 3, RMD209
Bedford Park5042
South Australia, Australia
Telephone: 61438650722
For: Breath Analysis (13 C sugar breath test) for gut health

Dr. Justine Chileshe (PhD)
Head of Biomedical Sciences Department/Senior Scientific Officer,
Tropical Diseases Research Centre
7th floor Ndola Teaching Hospital
P.O. BOX 71769 Ndola, Zambia
Mobile: +260-965-595-262
chileshej@tdrc.org.zm
For: Saliva analysis for body composition

Dr. Margaret N. Kosek
University of Virginia
328 Crispell Dr. MR-6 rm 2707
Charlottesville, VA 22908-0075
Mkosek@virginia.edu
Office (Virginia): 434 983 6768
Office (Peru): 5116524250
Cell: 443-900-7269
For: Urine Analysis (Lactulose Ramnose ratio for health)

You are directed to:

1. Provide a study progress update every six months until completion of the study to dhealthpolicy.research.kenya@gmail.com using the attached template.
2. The first such report is expected on or before **30th June, 2022.**
3. Ensure that the biological materials being exported are used solely for the analysis documented in the approved study protocol.
4. Submit the final study report to this office.

Note that this approval applies to this request only.

Dr. Patrick Amoth, EBS
Ag. DIRECTOR GENERAL FOR HEALTH

ISO 9001:2015
certified

Appendix XI: Pharmacy and Poisons Board Permit-Australia

REPUBLIC OF KENYA PHARMACY AND POISONS BOARD

EXPORT PERMIT

Document	321J - EXPORT PERMIT
Document Type	2 - Permit
Application Reference No : 2022CPPB321J0000607170	Version No : 1
Master Approval No	
Master Approval Version No	
UCR Number	UCR2200223203

Application Status

Approval Status :Approved - Pending cargo release	Application Date :2022-05-13 15:02:31.351	Amended Date :
Expiry Date :2023-05-13 17:12:50.808	Approval Date :13/05/2022 17:13:13	

Applicant Details

Name :SILETO PHARMACEUTICALS LIMITED

PIN :P051423018N	Application Code :Sll
Address :48694 00100	Country :KENYA
Contact Person :NA NA	Email : rkotut@gmail.com

Consignee Details

Name :Flinders Medical Centre

PIN :P000000000N	OGA Ref No :
Physical Address :South Australia	Physical Country :AUSTRALIA
Postal Address :	Postal Country :
Telephone :61438650722	Fax :61438650722
Email :rogeryazbek@gmail.com	Sector of Activity :
Warehouse Code :	Warehouse Location :

Importer Details

Name :Flinders Medical Centre

PIN :P000000000N	OGA Ref No :
Physical Address :South Australia	Physical Country :AUSTRALIA
Postal Address :	Postal Country :
Telephone :61438650722	Fax :61438650722
Email :rogeryazbek@gmail.com	Sector of Activity :
Warehouse Code :	Warehouse Location :

Item Details

Item No :1

Item Description :BREATH ANALYSIS 13C FOR GUT HEALTH SAMPLES) 4

Item HS Code :3002900000	HS Description :HUMAN BLOOD; ANIMAL BLOOD PREPARED FOR THERAPEUTIC, PROPHYLACTIC OR DIAGNOSTIC USES; TOXINS, CULTURES OF MICRO-ORGANISMS (EXCLUDING YEASTS) AND SIMILAR PRODUCTS.		Quantity :13
Unit Of Quantity :kilogram	Package Type :Carton	Package Quantity :4	Foreign Currency Code :KES
Unit Price FCY :0.77	Total Price FCY :10.00	Unit Price NCY :0.77	Total Price NCY :10.00
Country Of Origin :KENYA	Item Net Weight :5 kilogram	Item Gross Weight :5 kilogram	

Transport Details

Mode Of Transport :4	Mode Of Transport Desc :Air transport
Shipment Date :20220513	Port Of Arrival :Smithfield
Port Of Departure :Jomo Kenyatta International Airport	Customs Office :NBO
Freight Station :JOMO KENYATTA INTERNATIONAL AIRPORT	Cargo Type Indicator :DB

Appendix XII: Pharmacy and Poisons Board Permit-USA

REPUBLIC OF KENYA PHARMACYAND POISONS BOARD

EXPORT PERMIT

Document	321J - EXPORT PERMIT
Document Type	2 - Permit
Application Reference No : 2022CPPB321J0000607055	Version No : 2Master Approval No
Master Approval Version No	
UCR Number	UCR2200223091

Application Status

Approval Status :Approved - Pending cargo	Application Date :2022-05-13 14:29:17.297	Amended Date :release
Expiry Date :2023-05-13 17:15:35.325	Approval Date :13/05/2022 17:15:26	

Applicant Details

Name :SILETO PHARMACEUTICALS LIMITED	
PIN :P051423018N	Application Code :Sll
Address :48694 00100	Country :KENYA
Contact Person :NA NA	Email : rkotut@gmail.com

Consignee Details

Name :University of Virginia	
PIN :P000000000N	OGA Ref No :
Physical Address :Charlottesville	Physical Country :UNITED STATES
Postal Address :	Postal Country :
Telephone :4439007269	Fax :4439007269
Email :mkosek@virginia.edu	Sector of Activity :
Warehouse Code :	Warehouse Location :

Importer Details

Name :University of Virginia	
PIN :P000000000N	OGA Ref No :
Physical Address :Charlottesville	Physical Country :UNITED STATES
Postal Address :	Postal Country :
Telephone :4439007269	Fax :4439007269
Email :mkosek@virginia.edu	Sector of Activity :
Warehouse Code :	Warehouse Location :

Item Details

Item No :1

Item Description :100* 2 URINE ANALYSIS

Item HS Code :3002900000

HS Description :HUMAN BLOOD; ANIMAL BLOOD PREPARED
Quantity :100FOR THERAPEUTIC, PROPHYLACTIC OR
DIAGNOSTIC
USES; TOXINS, CULTURES OF MICRO-
ORGANISMS(EXCLUDING YEASTS) AND
SIMILAR PRODUCTS

Unit Of Quantity :kilogram | Package Type :Carton | Package Quantity :2 | Foreign :KES

Currency CodeUnit Price FCY :0.10 | Total Price FCY :10.00 | Unit Price

NCY :0.10 | Total Price NCY :10.00Country Of Origin :KENYA | Item Net

Weight :5 kilogram | Item Gross Weight :5 kilogram

Transport Details

Mode Of Transport :4

Mode Of Transport Desc :Air transport

Shipment Date :20220513

Port Of Arrival :Stuart

Port Of Departure :Jomo Kenyatta International Airport

Customs Office :NBO

Freight Station :JOMO KENYATTA INTERNATIONAL AIRPORT

Cargo Type Indicator :DB