

**ASSESSMENT OF ENVIRONMENTAL ENTERIC DYSFUNCTION
(EED) IN HEALTHY AND UNDERNOURISHED CHILDREN-A
CROSSTALK BETWEEN EED AND STUNTING**

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**A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of
Master's in Life Sciences of the Nelson Mandela African Institution of Science and
Technology**

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ABSTRACT

Undernutrition affects 20% of children under five in the developing world. Stunting is a prevalent form of undernutrition. Global prevalence of stunting in 2019 was 21.4%, while current Tanzania national average is 34%. Ruvuma is one of the regions with highest prevalence of stunting (44%) in Tanzania. This dissertation involved studies on factors responsible for the high prevalence of stunting in Ruvuma and their association with environmental enteric dysfunction (EED) in children. The study included randomly and conveniently sampled children below 5 years of age between April 2019 – January 2020 who attended outpatient clinics at hospitals in Ruvuma. Among children in the cross-sectional survey, 46% were females and 45.6% were stunted, while the case-control study had 42% females, 78% stunted cases, and EED prevalence of 57%. Through bivariate analysis, stunting was associated with gender ($\chi^2 = 6.6759$, $df = 1$, $p = 0.009772$), hand washing before food ($\chi^2 = 5.1213$, $df = 1$, $p = 0.02363$), location of hospital ($\chi^2 = 3.851$, $df = 1$, $p = 0.04972$) and use of Municipal garbage collection system ($\chi^2 = 3.6814$, $df = 1$, $p = 0.05502$). Moreover, diarrhea was associated with toilet sharing ($\chi^2 = 5.4703$, $df = 1$, $p = 0.002$), use of household's toilet ($\chi^2 = 4.0224$, $df = 1$, $p = 0.004$) and rinsing child feces into toilet ($\chi^2 = 3.6814$, $df = 1$, $p < 0.01$). Multivariate logistic regression analyses showed that, stunting risk increased with male gender (OR (95%CI) = 1.7945 (1.1944 – 2.712), age (OR (95%CI) = 1.3122 (1.1484 – 1.507), and decreased with hand washing before meal (OR (95%CI) = 0.5403 (0.3042 – 0.940). Finally, diarrhea risk increased with toilet sharing (OR (95%CI) = 2.154 (1.153 – 3.953) and decreased with child's use of toilet (OR (95%CI) = 0.510 (0.259 – 0.945). Our study generally revealed important factors that determined observed high prevalence of stunting in Ruvuma. These factors can be modified through health interventions to reduce the observed high prevalence. Nevertheless, the association between EED and stunting is undeniable and needs further assessment in a bigger study.

DECLARATION

I, Grantina Modern do hereby declare to the Senate of Nelson Mandela African Institution of Science and Technology that this dissertation is my original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

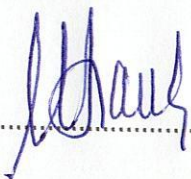
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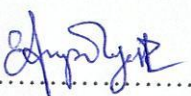
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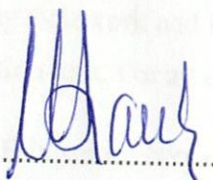
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CERTIFICATION

The undersigned certify that they have read the dissertation titled "*Assessment of Environmental Enteric Dysfunction (EED) in healthy and undernourished children-a crosstalk between EED and Stunting*" and recommended for examination in fulfillment of the requirements for the degree of Master's in Life Sciences of the Nelson Mandela African Institution of Science and Technology.

Dr. Elingarami Sauli Nkya

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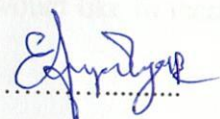
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DEDICATION

I dedicate this work to my beloved daughter, Gianna, for she is a blessing and motivation and my Mother as an appreciation of her effort and support when I needed it most.

TABLE OF CONTENTS

ABSTRACT.....	i
DECLARATION	ii
CERTIFICATION	iv
ACKNOWLEDGEMENTS	v
DEDICATION	vi
LIST OF TABLES.....	x
LIST OF FIGURES	xi
LIST OF APPENDICES.....	xii
LIST OF ABBREVIATIONS AND SYMBOLS	xiii
CHAPTER ONE	1
INTRODUCTION	1
1.1 Background of the Problem.....	1
1.2 Statement of the Problem	2
1.3 Rationale of the Study	3
1.4 Research Objectives	3
1.4.1 General Objective	3
1.4.2 Specific Objectives	3
1.5 Research Questions	4
1.6 Significance of the Study	4
1.7 Delineation of the Study.....	4
CHAPTER TWO	6
LITERATURE REVIEW	6
2.1 Known Stunting Interventions	6

2.2	Water, Sanitation and Hygiene.....	6
2.3	Environmental Enteric Dysfunction Pathogenesis.....	7
2.4	Impact of Environmental Enteric Dysfunction on Children	8
2.5	Environmental Enteric Dysfunction Biomarkers	9
2.6	Current focus of this study	10
CHAPTER THREE		12
MATERIALS AND METHODS.....		12
3.1	Ethical Consideration	12
3.2	Study Setting and Duration	12
3.3	Study Design	12
3.3.1	Assessment of WaSH and Stunting	12
3.3.2	Environmental Enteric Dysfunction	13
3.4	Sample Size	13
3.4.1	Cross-sectional Survey Study for WaSH and Stunting	13
3.4.2	Case-control Study for Environmental Enteric Dysfunction.....	14
3.5	Recruitment Procedure (Inclusion and Exclusion Criteria)	14
3.6	Data and Sample Collection.....	15
3.6.1	WaSH and Stunting	15
3.6.2	Environmental Enteric Dysfunction	16
3.7	Environmental Enteric Dysfunction Markers Investigation (Sample Analysis)	17
3.7.1	Determination of Lactulose Absorbance and Concentrations	17
3.7.2	Determination of Mannitol Absorbance and Concentrations	17
3.7.3	Calculations	17
CHAPTER FOUR.....		19
RESULTS AND DISCUSSION		19

4.1	Results	19
4.1.1	Water, Sanitation, Hygiene and Stunting	19
4.1.2	Environmental Enteric Dysfunction	26
4.2	Discussion	32
4.2.1	Water, Sanitation, Hygiene and Stunting	32
4.2.2	Environmental Enteric Dysfunction	35
CHAPTER FIVE		38
CONCLUSION AND RECOMMENDATIONS		38
5.1	Conclusion.....	38
5.2	Recommendations	38
REFERENCES		40
APPENDICES		54
RESEARCH OUTPUTS.....		62
	Journal papers.....	62
	Poster presentations	62

LIST OF TABLES

Table 1:	Demographic and Nutrition status summary	20
Table 2:	Cross-tabulations and chi-square tests for Stunting.....	21
Table 3:	Cross-tabulations and chi-square tests for diarrhea	22
Table 4:	Model output for status of Stunting.....	23
Table 5:	Odds ratio and their corresponding 95% CI for Stunting status.....	24
Table 6:	Model output for diarrhea status.....	25
Table 7:	Odds ratios and their corresponding 95% CI for Diarrhea status.....	25
Table 8:	Environmental Enteric Dysfunction and Stunting.....	27
Table 9:	Cross-tabulations and chi-square tests for EED	28
Table 10:	Model output for EED	29
Table 11:	Cross-tabulations and chi-square tests for simulated EED.....	31
Table 12:	Model output for simulated EED.....	32

LIST OF FIGURES

Figure 1: Relating poor WaSH, EED and Stunting	11
Figure 2: Demographic characteristics of the sample	27
Figure 3: Demographic characteristics of the simulated data	30

LIST OF APPENDICES

Appendix 1: Questionnaire	54
Appendix 2: Student Introduction letter from NM-AIST	58
Appendix 3: Ethical Clearance	60
Appendix 4: RMO - Ruvuma Research clearance	61

LIST OF ABBREVIATIONS AND SYMBOLS

Abbreviation	Description
AIDS	Acquired Immunodeficiency Syndrome
ART	Anti-Retroviral Therapy
DoB	Date of Birth
EED	Environmental Enteric Dysfunction
ELISA	Enzyme-linked Immunosorbent Assay
HIV	Human Immunodeficiency Virus
L:M	Lactulose: Mannitol
MAM	Moderate Acute Malnutrition
MUAC	Mid-Upper Arm Circumference
NACS	Nutrition Assessment, Counseling and Support
NM-AIST	Nelson Mandela African Institution of Science and Technology
OPD	Out Door Patient
PRH	Peramiho Mission Hospital
RCH	Reproductive and Child's Health
SAM	Severe Acute Malnutrition
SRRH	Songea Regional Referral Hospital
TDHS	Tanzania Demographic Health Surveillance
UNICEF	United Nations Children's Fund
WaSH	Water, sanitation and Hygiene

WHO

World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background of the Problem

Chronic undernutrition affects 25% of children (one in every four children) in the developing world and has been associated with half of all deaths worldwide in children aged less than 5 years of age and makes children vulnerable to diseases (Budge *et al.*, 2019a). The known forms of chronic undernutrition include wasting (low weight-for-height), stunting (low height-for-age $HAZ \leq -2$), deficiencies in vitamins and minerals and underweight (low weight-for-age) (World Health Organization, 2006).

Stunting is the most common and prevalent form of chronic undernutrition (Campisi, & Bhutta, 2017) that highly contributes to Tanzania's poverty (Budge *et al.*, 2019a). It affects approximately 165 million children globally, equivalent to 23% of all children below the age of five (approximately one in every four children) (Marie, *et al.*, 2018). The long-term effects of stunting in early childhood have been associated with cognitive and physical growth deficits, suppressing immunity, causing infections, and reducing the efficacy of childhood vaccines, poor school performance and reduced adult capacity (Mbuya & Humphrey, 2016).

There have been many efforts to fight chronic undernutrition (stunting) in African countries (Remans *et al.*, 2011). However, models depicting use of all known interventions to tackle undernutrition, including vitamin A and zinc supplementation, balanced energy protein supplementation, complementary feeding, breastfeeding promotion and micronutrient supplementation in pregnancy, have shown that their use in 99% of children would only decrease stunting by 33% worldwide, which clearly indicates that there is still a knowledge gap in our understanding of growth stunting and its other associated causal factors that cannot only be explained by mycotoxins (Smith *et al.*, 2015) and food insecurity, as commonly known (Harper *et al.*, 2018).

Environmental Enteric Dysfunction (EED) is an acquired subclinical condition caused by recurrent fecal-oral contamination, with resultant chronic intestinal inflammation and villous blunting (Campbell *et al.*, 2017). Fecal-oral contamination from environments with poor water, sanitation, and hygiene (WaSH) usually expose children directly or indirectly to entero-

pathogens such as bacteria, virus, protozoa or/and parasites, including but not limited to; *E.coli*, *Salmonella spp*, *Shigella spp*, *Rotavirus*, *Campylobacter spp*, *Entamoeba histolytica* etc (George *et al.*, 2018; Lifschitz & Sieczkowska, 2018). Enteric pathogens and their potential role in causing undernutrition have been a focal point for research. Pathogens may be introduced early in life and progression of infection consequently damage the intestinal mucosa of the child and marks the onset of EED syndrome (Julian, 2016; Singh *et al.*, 2021). Enteric infections damage intestinal villi that in return affect permeability and absorptive capacity of the intestine for micro and macronutrients essential for a child's growth (Budge *et al.*, 2019b). Both micronutrient deficiencies and chronic immune stimulation (that is immune response towards intestinal infection such as diarrhea and intestinal inflammation) have also been found to impair growth and increase susceptibility to infectious diseases (Blanton *et al.*, 2016). This study thus assessed the link between EED and stunting in children and investigated the possible factors responsible for the high prevalence of stunting in Songea, Ruvuma through assessment of WaSH services in selected hospitals of Songea, in order to possibly address new nutritional interventions that may work when dealing with stunting.

1.2 Statement of the Problem

Stunting is a problem known to be caused by many factors. Usually, stunting has been associated with food insecurity and failure to take into account other factors, such as environments with poor WaSH services. Moreover, many efforts have been put in place to counteract the stunting problem. For example, recent interventions in Tanzania have been known to address this problem through food security and safety measures only, including micronutrient and food supplementations. Regardless of the efforts put forward, these interventions have indicated limited efficiency and effect in reducing stunting prevalence, thus suggesting a need for combined approach by addressing other possible causative factors for stunting. In addition, diarrhea has been successfully treated in children but cases of malnutrition and stunting still persist, highlighting presence of other possible underlying environmental causes. However, very few studies have been conducted in Tanzania to directly associate stunting with EED as a possible underlying cause, instead most of studies have focused on food insecurity as the main cause. To overcome stunting, interventions have to also target all possible causes of stunting, which may also include dealing with EED. This study therefore assessed the link between EED, WaSH services and stunting in children from

Songea, Ruvuma, in order to possibly suggest new nutritional interventions that may help reduce stunting prevalence in Tanzania.

1.3 Rationale of the Study

Stunting has been shown to be a cause of poor health in children below five years of age globally, including Tanzania. Its prevalence is increasing locally due to continued exposure of children to poor WaSH environments.

Environmental Enteric Dysfunction is a causal factor that may also be associated with stunting in developing countries, hence studying the linkage between stunting and EED will widen the knowledge in our understanding of other related causal factors for stunting apart from food insecurity. Findings from this study will also provide information that can be adapted by healthcare policy makers to improve the existing stunting interventions in Tanzania.

1.4 Research Objectives

1.4.1 General Objective

To assess environmental enteric dysfunction (EED) in healthy and stunted children below five years of age in a selected study population in Ruvuma.

1.4.2 Specific Objectives

- (i) To determine nutrition status of children (below 5 years of age) and related factors of stunting.
- (ii) To determine EED prevalence in undernourished children using the lactulose/mannitol biomarker test.
- (iii) To determine the association between Stunting, WaSH, and EED among children in Songea.

1.5 Research Questions

- (i) What is the nutrition status of index child and related factors for stunting in children below age of five years of age in the selected population of Ruvuma, Tanzania?
- (ii) What is the prevalence of EED in selected children population from Ruvuma, Tanzania?
- (iii) Is there any association between Stunting, WaSH, and EED among children in Ruvuma, Tanzania?

1.6 Significance of the Study

There is currently a scarcity of evidence on specific functional changes of small bowel of children in resource-limited settings (like Tanzania) where environmental hygiene is generally poor. These changes are termed together as EED, a subclinical disorder that is asymptomatic in most cases. This study therefore will highlight on EED and its impact after comparative assessment of the study groups.

Environmental Enteric Dysfunction is marked by increased intestinal permeability, impaired gut immune function, mal-absorption, growth faltering, and oral vaccine failure, causing a significant impact on health status, and hence undernutrition due to limited supply of micronutrients for growth and development. This study, therefore, assessed the impact of EED on the nutrition status in healthy and undernourished children below the age of five from Ruvuma population in Tanzania.

A better understanding of the role of EED in malnourished children, including healthy and undernourished children less than 5 years of age, may help to introduce effective interventions which will improve the immune system, as a result causing children to respond more effectively to infectious challenges and orally administered vaccines and anti-retroviral therapy (ART).

1.7 Delineation of the Study

This study involved conducting a field survey, individual interviews and laboratory analysis to assess prevalence of EED and stunting in healthy and undernourished children from

Ruvuma below five years of age. The field survey involved sociodemographic and WaSH data collection and anthropometric measurements through a semi-structured questionnaire embedded in a kobo-collect toolbox. Also, it involved urine sample collection for EED marker investigation in the studied population.

Lack of a reasonable number of significant explanatory variables (5 out of 30 variables for stunting and 3 out of 30 variables for diarrhea), in this study may have been attributed by a number of reasons. First, possibility of recall bias is high with cross-sectional study designs. Therefore most of the provided information from the interviewee could be incorrect in significant ways such as for water treatment and hand washing habits. Another reason may have been due to the inherent cross-sectional design of the study leading to highly correlated information among variables, a follow-up design would probably have shown temporal changes in such information. Lastly, the two hospitals studied are not far from each other, and so there may be a spatial effect in the sense that actually for most variables the responses between the two locations are somewhat similar, a more spatially heterogeneous design might therefore be an improvement in the future.

The main linkage between EED and stunting in this study is through poor WaSH services and presence of infections (e.g. diarrhea), hence a need for further bigger study on this. Lack of information on available WaSH services and prevalence of diarrhea among the study population was the major limitation to this part of study, which may explain lack of association between EED and stunting in the above reported results.

Delay of this study was due to lack of research funds by then, delayed and prolonged procurement of analysis lactulose-mannitol ratio kit from the USA, Covid -19 lockdown and my maternity leave.

CHAPTER TWO

LITERATURE REVIEW

2.1 Known Stunting Interventions

In recognition of undernutrition (stunting) as a major problem, many interventions were put in place in Tanzania. In 2011, Tanzanian government put forward a five years national nutrition strategy of 2011/12 to 2015/16 to overcome all forms of undernutrition, including stunting. The strategical interventions included: infant and young child feeding, vitamin and mineral supplements, maternal and child malnutrition, assessing nutrition and HIV and AIDS, supporting children, women and households in difficult circumstances, educating on diet-related non-communicable diseases, household food security, nutrition surveillance, surveys and information management. The report also showed a limited efficiency of the efforts: reducing stunting from 44% to 35% only. Tanzania Demographic Health Surveillance (TDHS) reported a slight decrease in stunting from 35% to 34% in 2015/16: that is, affected one in every three children (Ministry of Health [MoH-Z], 2015).

Breastfeeding has healthy benefits to both mother and child, including but not limited to protection against infections and diseases such as upper and lower respiratory tract infections (Allen & Hector, 2005). Mother's milks have been proven to have immunological and antibacterial properties, as well as natural antibodies that protect the child (Oddy, 2001). Risks for stunting is highly reduced in breastfed and exclusively breastfed children, thus improving the cognitive development of children (Binns *et al.*, 2016). Breastfeeding promotion is one of important interventions in controlling diarrhea, which in turn leads to prevention of stunting and EED (De-Zoysa *et al.*, 1991).

2.2 Water, Sanitation and Hygiene

In all attempts to eradicate stunting, the importance of access to safely managed water sources within the premises and availability at all times for purposes of drinking and other house chores is paramount (World Health Organization, 2018). Moreover, safely managed sanitation services with unshared latrine facilities within the premises are also vital (United Nations International Children's Emergency Fund [UNICEF], 2016; Colston *et al.*, 2020). People, including children, should wash their hands with soap and water all the time within their

premises (Ejemot-Nwadiaro *et al.*, 2020). These improved conditions can prevent disease transmission (Guo *et al.*, 2018) that can lead to EED, which can later lead to stunting among children. Enteric pathogens can be harbored within the environment with poor WaSH and infect a susceptible host who may be a child below five years of age (Cumming & Cairncross, 2016). Thus, improved WaSH can prevent the transmission of enteric pathogens to a child (Humphrey, 2009).

2.3 Environmental Enteric Dysfunction Pathogenesis

Environmental Enteric Dysfunction has gained increasing interest as a key pathway linking unsanitary living conditions with different forms of undernutrition (i.e. stunting, wasting, and underweight) (Kosek *et al.*, 2014). The main causative factor for EED is exposure to fecal microorganisms through poor water, sanitation and hygiene (WaSH) (Ngure *et al.*, 2014). Other causative factors that may be important in the pathogenesis of EED include; mycotoxin exposure (Etzel, 2014), severe nutritional deficiency, and diarrhea.

Enteric pathogens and their potential role in causing undernutrition have been a focal point for research. Pathogens may be introduced early in life and may damage the absorptive capacity of the intestine, causing protein-energy and micronutrient malnutrition (Crane, *et al.*, 2015). Enteric infections, for example diarrhea, can compromise the intestinal barrier and increase intestinal inflammation (Briend, 1990), leading to decreased function (Syed *et al.*, 2016). Moreover, a chronic infection that causes chronic immune activation may result in absorbed nutrients (e.g. amino acids) being diverted towards inflammatory processes instead of being used as building blocks or for serving their metabolic functions (Schiffrin & Blum, 2002).

A chronically damaged and inflamed gut may increase the likelihood of developing a bodily predisposition to becoming undernourished through several pathways; while being undernourished further compromises healthy intestinal lining, thus increasing susceptibility to additional infection (Velly *et al.*, 2017; Chen *et al.*, 2020). Chronic intestinal inflammation, a symptom of EED, has been associated with altered absorption and ineffective utilization of micronutrients and macronutrients (Owino *et al.*, 2016). Chronic diarrhea comes as a result of chronic intestinal infection which in turn may result in chronic undernutrition as a consequence (Guerrant *et al.*, 1992). In lower and middle-income sub-Saharan countries, chronic diarrhea in children less than 5 years of age is a second leading cause of death. It has a

mortality of about 760 000 annually, in Tanzania the prevalence of diarrhea in children below five years of age is 12.1% (Edwin & Azage, 2019). Chronic malnutrition is the major cause of stunting in children less than 5 years of age (Richard *et al.*, 2013).

2.4 Impact of Environmental Enteric Dysfunction on Children

It has been hypothesized that; the failure of health interventions that specifically target malnutrition can directly be attributed to EED. One of the most significant impacts of EED may be causing of undernutrition and potentially growth faltering in children. Moreover, EED potentially contributes to poor response to nutrition therapy, paving way for secondary effects such as diarrhea and stunting, which increase child morbidity, mortality, and reduce cognitive development and adult economic productivity (Denno *et al.*, 2016; Dewey & Begum, 2011).

Some impacts of EED on children include; impairment of growth and development, neurodevelopment, and oral vaccine response (Watanabe & Petri, 2016), all resulting from lack of adequate micro and macronutrients essential for the body (Harper *et al.*, 2018). Although the syndrome is not fatal, it is a major setback especially in growth and development of children within their first two years, thus causing stunting.

This syndrome (EED) is said to pose interference with oral medication and vaccines efficacy. Oral vaccines have been observed to become less immunogenic in children of low and middle-income countries (Parker *et al.*, 2018). Among many reasons for this is exposure to enteric pathogens and environmental enteropathy, i.e. EED, which is hypothesized to occur in them (Vlasova *et al.*, 2019). Currently, the impact of EED is observed on live-attenuated rotavirus vaccines (all three serotypes) (Harris *et al.*, 2016). The vaccine is administered to infants starting from six (6) weeks, with next dose varying depending on the serotype used (World Health Organization, 2009).

Environmental Enteric Dysfunction also impairs the immune system, increasing child's susceptibility to infections and diseases. Although a review done by James Church in 2018 showed no biological link between EED and oral vaccine (Church *et al.*, 2018), few studies have shown positive correlation (Marie *et al.*, 2018). Increased intestinal permeability poses an adverse effect on metabolic pathways; as Richard Semba and others (2016) reported its contribution to stunting. The study revealed alteration of gut function and integrity due to abnormalities of metabolic pathways, such as dominant phosphatidylcholines in the cell

membrane for cell growth and development in early development of a child (Semba *et al.*, 2016).

2.5 Environmental Enteric Dysfunction Biomarkers

Lactulose to mannitol ratio (L:M) has been the most commonly used biomarker for EED. This test is commonly used as a marker for intestinal mucosal permeability. Lactulose is a large sugar that is not normally absorbed by the small intestine, while Mannitol is a smaller sugar that is absorbed by the small intestine in proportion to absorptive surface area. With an altered permeability, both lactulose and mannitol pass the mucosa unabsorbed after an oral ingestion of disaccharide solution, and later excreted intact in the urine following minimal metabolism. Urinary mannitol in the urine indicates an index of absorptive capacity, while lactulose in the urine indicates impaired barrier function. Although the method has been effective, there has been less correlation of L: M test with EED (Singh *et al.*, 2020). A potential reason for this might be lack of local controls to the test for comparison, this suggesting that, other methods are relevant to support these investigations (Keusch *et al.*, 2014).

Environmental Enteric Dysfunction can also be investigated using a number of protein biomarkers released within the body. Neopterin is a protein that indicates intestinal inflammation. Calprotectin is also a neutrophil protein found in both stool and blood plasma (Konikoff & Denson, 2006). Fecal calprotectin is a protein in stool that is used as a marker to detect intestinal inflammation. Increased levels of this marker in the stool or plasma indicate inflammation except in infants: which is due to normal physiology (D'Haens *et al.*, 2012). Myeloperoxidase is an enzyme of neutrophils' granules and lysosomes of monocytes, which are antimicrobial proteins. It is found in high concentration within young neutrophils and lower concentrations in monocytes and macrophages (Goosmann *et al.*, 2014). Alpha 1 antitrypsin is also a protein released by neutrophils that can also be found in the stool, urine or serum. Its presence in stool indicates increased permeability from the blood into the gut lumen and protein loss. It has an inhibitory function against protease due to inflammation-causing infections (Arndt *et al.*, 2016). Fecal lactoferrin and Lipocalin-2 are fecal proteins that biomarkers for intestinal inflammation (Prata *et al.*, 2016). Serum metabolites, such as phosphatidylcholines, citrulline, ornithine, glutamate, and serotonin were suggested in 2016 by Richard as biomarkers for EED when investigating gut integrity. These biomarkers showed positive correlation with EED in the study conducted in Malawi (Semba *et al.*, 2016).

In the course of establishing non-invasive methods for diagnosis of EED, Ordiz established EED diagnosis using fecal transcripts. The study worked with and suggested fecal mRNAs as non-invasive EED biomarker (Yu *et al.*, 2016). Additionally, fecal mRNA can be used by machine learning model to predict EED with more than 80% sensitivity (Ordiz *et al.*, 2016).

Recently, the use of serum antibodies IgA and IgG as indicators of bacterial antigens associated with risk for EED has been acknowledged. Flagellin and Lipopolysaccharides are antigens and biomarkers for bacterial translocation; which have been associated with EED, and thus higher risk for stunting. The novel anti-flagellin and anti-lipopolysaccharide are serum antibodies to be used as biomarkers for EED (Locks *et al.*, 2017; Syed *et al.*, 2018).

2.6 Current focus of this study

To prevent stunting, we need to prevent the onset of EED because: (a) EED is self-perpetuating once it has developed (b) recovery from EED is relatively slow even when there is a dramatic change in environment; and (c) the window for critical growth and development is short (between conception and the first two years of postnatal life). In the context of marginal diets due to poverty and recurrent infections in developing countries like Tanzania, EED likely explains a significant portion of the unresolved stunting that affects one in every three children in these countries as investigated by Tanzania Health Demographic Surveillance in 2016 and, hence the focus for this research study as depicted in Figure 1.

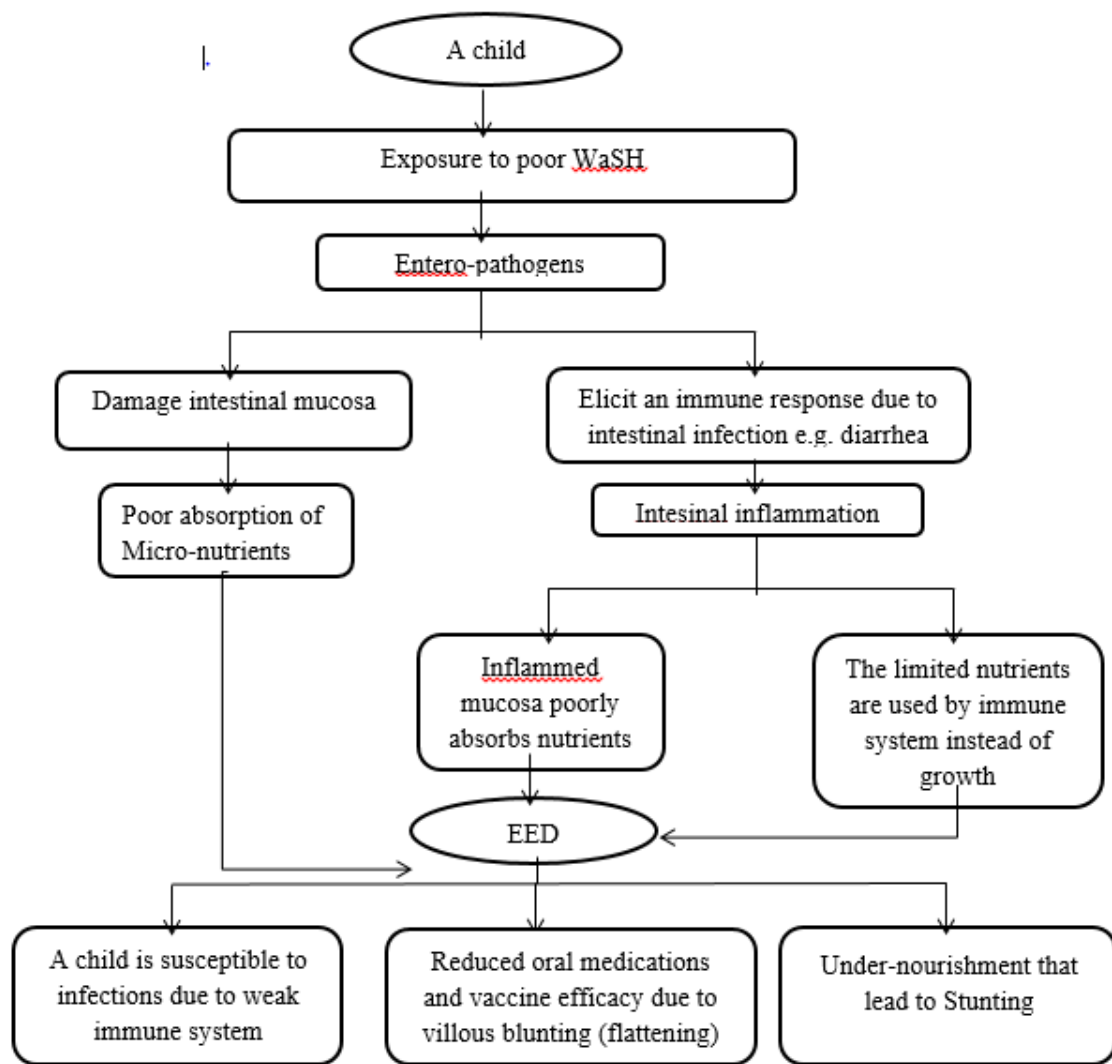


Figure 1: Conceptual framework relating poor WaSH, EED and Stunting

CHAPTER THREE

MATERIALS AND METHODS

3.1 Ethical Consideration

The study was approved by Kibong'oto National TB Hospital, Nelson Mandela African institution of Science and Technology, CEDHA Health Research Ethical Committee (KNCHREC) at Kibong'oto Research Centre (KRC) Kilimanjaro, Tanzania. All children's parents and/or guardians were informed about the study and gave their informed consent.

3.2 Study Setting and Duration

These studies were conducted in the South of Tanzania in Ruvuma region. Ruvuma was selected for its high prevalence of stunting (44%) in children between 7 months and 5 years of age. Also, the region is one of the regions in Tanzania with secured food production and yet has a high prevalence of stunting. This raised curiosity that, stunting in Ruvuma may be associated with other factors like EED apart from food insecurity, as it is commonly known.

The general study was conducted in one year, starting from January 2019, and the aim was to assess factors associated with stunting, poor WaSH and EED. The cross-sectional study was conducted from April to May 2019 and case-control study was conducted in January 2020.

The data collection centers for the study area were health care facilities. Two districts, Songea Urban and Songea rural districts had been selected with a total of two (2) health care facilities namely Songea regional referral Hospital and St. Joseph Mission Hospital Peramiho for sample selection. The selected hospitals increased chances of capturing cases in both rural and urban areas. Also, compared to other hospitals, these had high number of children below the age of 5 years attending Reproductive and Child Health (RCH) Unit.

3.3 Study Design

3.3.1 Assessment of WaSH and Stunting

This part involved a hospital-based cross-sectional study to assess and analyze the exposure of children to poor water, sanitation and hygiene, as factors associated with stunting among children between 7 months and five years of age. The survey study was conducted using

questionnaires, which were administered through an interview. The study was hospital-based because most of patients with diarrhea tend to seek care in the hospitals thus facilitating easiness for cooperation with patients when in a hospital environment, as well as minimizing movements from household to household, which would have implications in the monetary aspect of the study.

3.3.2 Environmental Enteric Dysfunction

This part of study was a hospital-based retrospective case-control study that assessed the impact of EED on growth in both undernourished/stunted (Height for Age Z-score ≤ -2 (HAZ ≤ -2)) and healthy/non-stunted (HAZ ≥ -1) children below the age of five in Ruvuma region of Tanzania. The control group was non-stunted children and case group was stunted.

3.4 Sample Size

3.4.1 Cross-sectional Survey Study for WaSH and Stunting

Sample size was determined using the following formula for cross-sectional survey in (Kothari, 2004).

$$n = \frac{deff}{r} \times \frac{Z^2 \times P(1 - P)}{e^2}$$

Where; n= minimum sample size (for population $>10\ 000$), Z= level of significance, 1.96 at 95% Confidence level, deff is design effect, 1, r is 0.9, P is 0.5 and e= 0.05 (usually as a proportion, 0.05 for 5%). Thus, a minimum number (n) of 426 children were randomly selected as the sample size of the study for assessment of poor WaSH and Stunting.

The minimum number of each stratum (health care facility) n_i was determined using the following formula; for stratum 1 (Songea Regional Referral Hospital (SRRH)): $n_1 = \frac{n}{(Y_1+Y_2)} \times Y_1$, for stratum 2 (Peramiho Mission Hospital (PRH)): $n_2 = \frac{n}{(Y_1+Y_2)} \times Y_2$, thus $\sum n_i = n$ (sample size i.e. 426): Y being the average children population per health facility. From SRRH, y_1 was 11 176 children and from PRH, y_2 was 32 671 children giving a total of 43 847 children. Thus $n_1 = \frac{426}{(43\ 847)} \times 11\ 176 = 108.58 \sim 109$ and $n_2 = \frac{426}{(43\ 847)} \times 32\ 671 = 317.42 \sim 317$. From, $\sum n_i =$: thus $109 + 317 = 426$.

3.4.2 Case-control Study for Environmental Enteric Dysfunction

Sample size was determined using the formula for case-control design below:

$$n = \frac{(r + 1)}{r} \times \frac{(SD)^2 (Z_{\beta} + Z_{\alpha/2})^2}{\delta^2}$$

Where; n is the minimum sample size in case group, r is ratio of case to control, set at 1:1, SD is the standard deviation, 3, Z_{β} is the desired power of study (typically 0.84 for 80% power), $Z_{\alpha/2}$ is the level of significance of 1.96 at 95% confidence interval (CI), δ is margin of error (i.e. SD/2), 1.5. Therefore, $n = 62.72 = 63$. Thus, a minimum number of 63 stunted children were conveniently selected as cases and 63 normal (non-stunted) children were selected as controls for 1:1 ratio which was deduced from the poll of 426 children for downstream assessment of EED syndrome.

3.5 Recruitment Procedure (Inclusion and Exclusion Criteria)

For both studies, from selected two districts, a total of two (2) health care facilities; namely Songea regional referral Hospital and St. Joseph Mission Hospital Peramiho were visited. The reproductive and child's care units (RCH) in both hospitals were the fieldwork areas. Both hospitals had clinic visit scheduled for every workday from Monday to Friday from 8:00 am to 3:30 pm. The study targeted every child below five years of age who attended the hospital for the purpose of charting or OPD cases.

Upon encounter of a child's parent(s) or guardian(s) during the above stated working hours, the studies were explained. Those willing to participate were recruited and provided with an informed consent form. An inclusion criterion was being a child below 5 years of age and willing to participate, while exclusion criteria were children above 5 years of age, critical patients and those below 5 but not willing to participate. No follow up was done because of the study design but severely wasted cases were reported to the unit in charge and advised for follow up to avoid mortality.

3.6 Data and Sample Collection

3.6.1 WaSH and Stunting

(i) Nutrition Status of Index Child

Anthropometric measurements (height/length, weight, and mid-upper arm circumference) were taken using tools, such as height ruler, weighing scale and MUAC tape measure, respectively for children below the age of 5 years, to determine their nutritional status using currently available WHO growth standards. All measurements were measured as per Tanzania NACS job Aids of 2016. These included:

Date of birth (DOB): The date of birth for each child was inquired from the caretaker/ mother and cross-checked from immunization/growth cards and recorded in months and years.

Length/ height: Length for children below five years was measured by lying flat and centrally on measuring boards placed on a hard-flat surface on the ground. The length was read to the nearest 0.1 cm (head and feet against the base of the board and foot piece respectively).

Weight: Children were weighed to the nearest 0.1 kg by using ideally an Electronic SECA Scale. The children who could easily stand were asked to stand on the weighing scale and their weights were recorded. In a situation where the children could not stand up, balance-hanging weighing method was applied.

MUAC: Mid Upper Arm Circumference for the children below five years was measured using MUAC tapes to the nearest of 0.1 cm for children below five years of age. The tape indicated a range of growth status that read green for normal, yellow for moderate acute malnutrition and red for severe acute malnutrition.

(ii) Socio-demographic Data

A semi-structured questionnaire administered through interviews to parents/guardians of the selected children was used to obtain socio-demographic characteristics for the study population. The questionnaire also incorporated anthropometry, water availability, hygiene, and sanitation. These data were collected and stored using KoBo Tool Box designed as KoBoCollect v1.14.0a. This was done at RCH unit of each hospital. Principal investigator and one field assistant from each hospital who was either a nurse or medical attendant did

anthropometry measuring and questionnaire interviewing. The principal investigator closely supervised the field assistants.

3.6.2 Environmental Enteric Dysfunction

(i) Laboratory Samples

This part of the study conveniently selected 126 children between 1 and 5 years from the cross-sectional study with a sample size of 430 children, for the case-control study, in order to investigate EED using urine samples. Using contact details from the questionnaires, parents/guardians of selected children were contacted to report back to the RCH Units of both Songea Regional Referral Hospital and St. Joseph Peramiho Mission Hospital. Since, the two phases of the study had a big time apart, when the selected 126 children's parents/guardians were contacted, many weren't reachable; assuming it was because by then Tanzania Communications Regulatory Authority (TCRA) had introduced fingerprint registration and most people changed phone numbers. Some had gone to their plantations/farms and some had travelled. Only less than 20 recruits were reachable and reported back. This led to new recruitment of children between 1 and 5 years, where we ended up with a total of 100-sample size instead of 126. The involvement of children between 1 and 5 years targeted easy urine collection. The procedure was then explained to the participants until they consented.

A 20 mLs disaccharide solution was first administered to the selected children followed by 300 mLs drinking water, then after 30 minutes to two hours; urine samples were collected using the 50 mLs urine containers/ universal bottles for lactulose-mannitol ratio test. The sample was collected only once for each child.

For some children, it took longer than 2 hours to void and had to administer the solution again and some parents complained of hunger and had to be permitted to leave without sample collection, which led to disqualification from study and prolonged field activity phase (time) than expected in search of more recruits.

Urine sample aliquots were mixed with three drops of Thimerosal as a preservative in cryovials to make a total of 2 mLs then stored in a freezer at a temperature of -20°C at Songea Regional Referral Hospital's laboratory awaiting transportation to NM-AIST laboratory for analysis.

3.7 Environmental Enteric Dysfunction Markers Investigation (Sample Analysis)

Determination of EED through the lactulose: mannitol test was conducted at NM-AIST laboratories. From Songea Regional Referral Hospital's laboratory, the frozen sample aliquots were transported to NM-AIST laboratories using a cool box and ice packs. Understanding that the cool box can only keep samples safe for less than 6 hours for samples at a temp of -20°C, ice packs were changed twice per day. Transportation using a private car from Songea paused at Dodoma in the evening and resumed the next day from Dodoma to Arusha. Upon arrival, all aliquots were stored in a freezer at a temperature of -20°C.

3.7.1 Determination of Lactulose Absorbance and Concentrations

Prior to the assay, sample aliquots and the analysis kit (lactulose-mannitol ratio test kit with all the used reagents, which was purchased from BioAssay Systems, USA: BioAssay Systems 3191 Corporate Place, Hayward, CA 94545, U. S. A) were equilibrated to room temperature. Enzyme A (dried) was reconstituted by adding 120 uL of enzyme buffer to the enzyme A tube. Then all kit reagents were briefly centrifuged at 13000 mps for 60 seconds before opening. Standard solutions and working reagents were prepared according to the SOP and then briefly vortexed. After adding the samples, standards and working reagents to the 96 well plate as per SOP, they were incubated in the dark for 60 minutes at room temperature. Optical densities were then read at 565 nm using an UV spectrometer and results were recorded in form of an excel sheet.

3.7.2 Determination of Mannitol Absorbance and Concentrations

Using the centrifuged mannitol reagents, standard solutions and working reagents were prepared. Samples, standards and working reagents were then added into a 96 well plate as per assay protocol and incubated for 30 minutes at room temperature. Optical densities were read at 565 nm using an UV spectrometer and results were recorded in the form of an excel sheet.

3.7.3 Calculations

Lactulose and mannitol individual absorptions were calculated as per protocol formula for each child. The ratio was simply obtained by dividing lactulose absorption to mannitol absorption. Higher urinary L: M ratios of > 0.05 reflected greater abnormalities of one or both

functions; as mannitol gives us an index of absorptive capacity, while lactulose indicate impaired barrier function thus EED.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Water, Sanitation, Hygiene and Stunting

(i) Demographic Characteristics

Out of 430 children, 109 were from rural Songea (25.3%) and 321 from urban Songea (74.7%). Also, 233 children (54.2%) were male while 197 (45.8%) were female as per Table 1 below. The average age of children in months was approximately 18 months while the median was 13 months. Moreover, out of 430 children, 24% had diarrhea and 65% of 24% diarrhea cases were treated. From the interviewed families, only 22.8% of male parents/guardians and 11.9% of female parents/guardians were employed.

Households that reported to have access to improved latrines (flush toilets) were 88.6% and 12.3% of all households shared toilets. About 65.8% had access to clean and safe drinking water (public tap and bottled water). There were 42.3% of the households that had water sources close to their premises, while 57.2% had them within the premises and 0.5% had to walk a long distance to a source. Also, households that treated water were 40.9% and specifically, 40.4% boiled while 0.5% used other treatment methods. This means that 59.1% drank untreated water running risks of water-borne infections. For garbage disposal, only 6.8% of households practiced safe disposal using the municipal garbage collection system and burning, while for child's fecal disposal, 84% of households practiced safe disposal and 15.6% of children used toilets. A high number of households, 96.3%, reported frequent hand washing (with/without soap), and specifically, 93.3% used soap during hand washing. Although lack of follow up, makes this information doubtful.

(ii) Nutritional Status

With respect to nutritional status, a total of 196 children (45.6%) were stunted while 234 (54.4%) had healthy nutritional status. Most stunted children were between 7 to 9 months of age. More male children (51.5%) were stunted than female children (38.6%). Stunting status, before being converted into a binary variable had three levels; moderately stunted, severely

stunted and normal. From the sample, 54.42% of children were normal, 24.65% were moderately stunted and 20.93 were severely stunted, see Table 1.

Table 1: Demographic and nutrition status summary

Variable		Level	Percentage (%)	Frequencies
Gender		Male	54.19	233
		Female	45.81	197
Age		7-12 months	49.07	211
		13-24 months	27.91	120
		25-36 months	10.00	43
		37-48 months	6.51	28
		49-59 months	6.51	233
		Severely stunted	20.93	
Stunting status		Moderate stunted	24.65	
		Normal	54.42	
Status/Gender	Male	120 (51.5%)		
Stunted	Female	76 (38.6%)	196 (45.6%)	
Not-stunted (Normal)	Male	113 (48.5%)		
	Female	121 (61.4%)	234 (54.4%)	
Total			430	

(iii) Bivariate Analyses for Factors Associated with Stunting

Cross-tabulations and chi-square tests were conducted to a total of 75 variables against stunting status.

Table 2: Cross-tabulations and Chi-square tests for Stunting

Variable (factor)	Levels	Status	
		Stunted	Not-stunted
Gender	Male	120	113
	Female	76	121
Chi-square results	X-squared = 6.6759	df = 1	p-value = 0.009772
District	Songea Rural	59	50
	Songea Urban	137	184
Chi-square results	X-squared = 3.851	df = 1	p-value = 0.04972
Garbage disposal through municipal collection system	No	189	214
	Yes	7	20
Chi-square results	X-squared = 3.6814	df = 1	p-value = 0.05502
Hand washing before food	No	172	185
	Yes	24	49
Chi-square results	X-squared = 5.1213	df = 1	p-value = 0.02363

* Hand washing before food for a person who prepares a child's meals and who feeds them

Out of 30 variables, only 5 variables were statistically significant in explaining stunting. Stunting seems to be dependent on gender, rural-poor versus urban-improved environment (here represented by District), use of the municipal garbage collection system and handwashing before eating food, as per the Table 2. It is fair to say that except for the p-values for gender, other p-values are only marginally significant.

(iv) Bivariate Analyses for Factors Associated with the Diarrhea Disease

Cross-tabulations and chi-square tests were also conducted to a total of 30 variables against diarrhea status of a child.

Table 3: Cross-tabulations and Chi-square tests for diarrhea

Variable (factor)	Levels	Diarrhea Status	
		No	Yes
Toilet sharing	No	294	83
	Yes	33	20
Chi-square results	X-squared = 5.4703	df = 1	p-value = 0.02
Child using toilet	No	254	90
	Yes	73	13
Chi-square results	X-squared = 4.0224	df = 1	p-value = 0.04
Rinsing child's feces into the toilet	No	68	9
	Yes	259	94
Chi-square results	X-squared = 6.9476	df = 1	p-value < 0.01

*Toilet sharing for more than one household

Out of 30 variables, only 3 variables were statistically significant to explain diarrhea. Diarrhea was dependent on whether households shared their toilets, whether a child used toilets versus random defecation, and whether the child's feces are rinsed into the toilet instead of being thrown elsewhere in the environment, see Table 3.

(v) Regression Analyses for Correlates of Stunting

For the model, 30 variables were used to formulate the logistic regression. In this case, the outcome variable was the status of stunting. Originally, stunting had three levels; normal, moderate and severe. Later decided to convert it into two categories; present and absent. A level of normal was called no stunting, while moderate and severe were combined and called stunting. Starting from the model with all explanatory variables, model selection procedures such as visualization of diagnostic plots and ANOVA tests were applied to come to the following logistic regression model:

$$\text{Stunting} = \text{alfa} + b1*\text{gender} + b2*\text{age} + b3*\text{handwashingbeforefood} + \text{error term}.$$

Where, alfa is the y-intercept, b1 the coefficient of gender, b2 the coefficient of age and b3 the coefficient of handwashing status before food. Finally, error term is the term that has random errors; which in this case are considered to be normally distributed with a mean of 0 and standard deviation of 1.

However, upon observing model diagnostics, there was a strong pattern in the residuals with respect to age (p-value < 0.001). This necessitated adjustment of the coefficient for age. A quadratic term was added and the model became:

$$\text{Stunting} = \text{alfa} + b1*\text{gender} + b2*\text{age} + b3*\text{age}^2 + b4*\text{handwashingbeforefood} + \text{error term}.$$

However, the model with a quadratic term still had some pattern with respect to age, and so a third-order term was added to age to produce the following model.

$$\text{Stunting} = \text{alfa} + b1*\text{gender} + b2*\text{age} + b3*\text{age}^2 + b4*\text{age}^3 + b5*\text{handwashingbeforefood} + \text{error term}$$

Model diagnostics summaries indicated this to be the final model to explain correlates of stunting. Therefore, used this model to present the results of the regression analysis, see Table 4:

Table 4: Model output for status of Stunting

	Estimate	Std error	z-value	Pr(> z)
Intercept	-2.53e+00	4.67e-01	-5.42	5.9e-08 ***
Gender (Male)	5.85e-01	2.09e-01	2.80	0.0051 **
Age in months	2.72e-01	6.92e-02	3.93	8.6e-05 ***
Age in months2	-8.03e-03	2.86e-03	-2.81	0.0050 **
Age in months3	7.14e-05	3.31e-05	2.16	0.0309 *
Hand washing before food	-6.16e-01	2.87e-01	-2.15	0.0318 *

*statistically significant

* Hand washing before food for a person who prepares a child's meals and who feeds them

Therefore, this study showed that, being a male gender increased the log odds for being stunted by 0.585, while a unit increase in age increased the log odds of being stunted by 0.272 for the first order of the age variable. The second order of polynomial for age was associated with decreased log odds of -0.00803 while the third order polynomial for age was associated with increased log odds for 0.0000714. Higher order terms for the age variable may not have a direct interpretation but they may point to the fact that the effect of age was only in the early childhood (less than 2) years where the function is usually linear (and hence the significance

and importance of the linear term). Hand washing before food was associated with decreased log odds for stunting by -0.616. In order to get epidemiologically intuitive information, the odds ratios together with their 95% confidence intervals were calculated as shown in Table 5 below:

Table 5: Odds ratio and their corresponding 95% CI for stunting status

	Odds ratios	Lower 95% CI	Upper 95% CI
Intercept	0.0794	0.0309	0.193*
Gender (Male)	1.7945	1.1944	2.712*
Age in months	1.3122	1.1484	1.507*
Age in months ²	0.9920	0.9864	0.998
Age in months ³	1.0001	1.0000	1.000
Hand washing before food	0.5403	0.3042	0.940*

*Significant odds ratios

Results from Table 5 show that, being a male gender was associated with increased odds for stunting by up to 80% compared to being a female gender. A unit increase in age seems to be associated with increased odds for stunting by 31% per unit age increase in the early years. However, the odds for stunting by age may not be significant throughout the age range of a human being as evidenced by lack of significance of odds ratios in coefficients of higher order polynomials. Hand washing before taking food is associated with a 46% decrease in odds of being stunted.

(vi) Regression Analyses for Correlates of Diarrhea

In a manner of model selection similar to the section above, this study made presence or absence of child diarrhea an outcome variable and arrived at the following final logistic regression model:

$$\text{Child diarrhea} = \text{alfa} + b1 * \text{toilet sharing} + b2 * \text{child uses toilet} + \text{error term}.$$

Where alfa is the y-intercept, b1 the coefficient of toilet-sharing status, b2 the coefficient of child use of toilet status and the error term is as define in the section above.

Therefore, in this study, child diarrhea is explained only by two factors: whether households share toilets and whether children in such households use toilets. The model outputs are shown in Table 6 below.

Table 6: Model output for diarrhea status

	Estimate	Std. error	Z value	Pr(> z)
Intercept	-1.142	0.134	-8.54	<2e-16 ***
Toilet sharing	0.767	0.313	2.45	0.014 *
Child uses toilet	-0.673	0.327	-2.06	0.040 *

*Toilet sharing for more than one household

While the results in these aspects were only marginally statistically significant, they offer interesting and useful pieces of information. Toilet sharing seems to increase the log odds for stunting by 0.767 while a child-using toilet tends to decrease the log odds for stunting by 0.673. Converting these results into a table of odds ratios and their corresponding 95% range produced the following Table 7.

Table 7: Odds ratios and their corresponding 95% CI for Diarrhea status

	Odds ratios	Lower 95% CI	Upper 95% CI
Intercept	0.319	0.244	0.413
Toilet sharing	2.154	1.153	3.953*
Child uses toilet	0.510	0.259	0.941*

*Toilet sharing for more than one household

More intuitively, toilet sharing more than doubles the odds for stunting among children while a child's use of toilet almost halves the odds of stunting among this population of children.

(vii) A Comment on Municipal Garbage Collection System

Participants in this study were asked about their use of the municipal garbage collection system. In both bivariate analysis against stunting ($\chi^2=3.6814$, $df=1$, $p=0.05502$) and in the multivariate logistic regression against diarrhea (OR (95% CI) = 0.448 (0.168 – 1.070, p -value = 0.084), it was only marginally statistically significant in the first instance and not statistically significant at all in the second instance. Instead of dropping it altogether this study shows that, it is an important variable and its marginal statistical significance warrants some attention. In this study only 6.8% of households used the municipal garbage collection

system, but then this small population enjoyed an almost statistically visible impact of diarrhea risk reduction and even a slight decrease in stunting (Table 2). It is believed that as this proportion of users of this garbage collection system increases, stronger evidence of its impact in diarrhea and stunting will be visible and that this study is a weak signal to that potentially positive impact. Municipal garbage collection system is one of the more systematic ways of garbage collection and potentially the cheapest public service system and this study indicates that it might work in reducing cases of diarrhea and stunting among the children in a resource-poor setting.

4.1.2 Environmental Enteric Dysfunction

(i) Demographic Characteristics

A sample size of 89 children with 6 variables; age in months, gender, underweight, wasting and stunting explaining EED syndrome, was incorporated into R software version 3.4 for univariate, bivariate and multivariate analysis.

Out of 89 children, 52 (58%) were male while 37 (42%) were female. The mean and median ages of children in months were 25 and 20 months, respectively. Out of all children, 57% were between the age of 12 to 24 months and amongst them, 57% had EED. While 57% of all children had EED, 78% of all cases were stunted. Moreover, out of 69 stunted cases, 57% had EED. In addition, from the sample size, 13% were underweight and only 2% were wasted as per figure 2 and table 8.

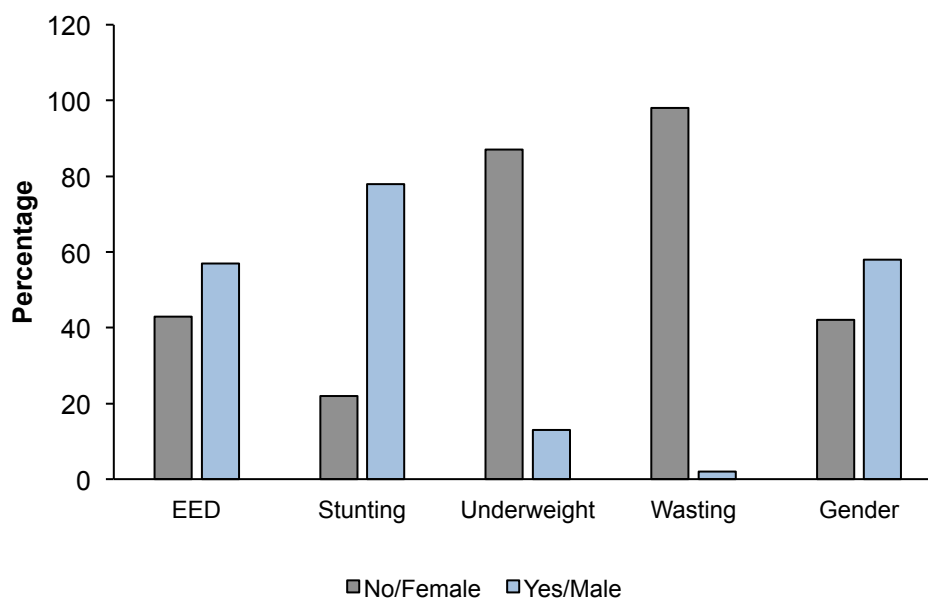


Figure 2: Demographic characteristics of the sample

Table 8: Environmental Enteric Dysfunction and Stunting

		Stunting		Total
		No	Yes	
EED	No	08	30	38
	Yes	12	39	51
Total		20	69	89
Age in Months		EED		
Age Group	No. of children			
		Yes	No	
12-24	51 (57.3%)	29 (57%)	22 (43%)	
25-36	25 (28.1%)	15 (60%)	10 (40%)	
37-48	08 (8.9%)	04 (50%)	04 (50%)	
49-60	05 (5.6%)	03 (50%)	03 (50%)	

(ii) Bivariate Analyses for Factors Associated with Environmental Enteric Dysfunction

Cross-tabulations and Chi-square tests were conducted to a total of 5 variables against EED and results were tabulated in table 9.

Table 9: Cross-tabulations and Chi-square tests for Environmental Enteric Dysfunction

Variable (factor)		Levels	EED	
			No	Yes
Stunting	No		08	12
	Yes		30	39
Chi-square results	X-squared = 0.00040766		df = 1	p-value = 0.9839
Underweight	No		34	43
	Yes		04	08
Chi-square results	X-squared = 0.15309		df = 1	p-value = 0.6956
Wasting	No		36	51
	Yes		02	0
Chi-square results	X-squared = 0.87262		df = 1	p-value = 0.3502
Gender	Male		22	30
	Female		16	21
Chi-square results	X-squared = 0		df = 1	p-value = 1
	Odds Value	2.5% CI	97.5% CI	P value
Y-Intercept	1.553260167	0.592793259	4.130697133	0.370745082
Age in months	0.994193384	0.960144339	1.029677645	0.740894058

No variable was statistically significant to explain EED after running bivariate analyses for the outcome variable EED with level Yes and No.

(iii) Regression Analysis for Correlates of Environmental Enteric Dysfunction

The study used the 5 non-significant variables from the bivariate analyses to formulate the logistic regression. The outcome variable remained being EED with levels yes and no. Level yes represented presence of EED syndrome, while level no represented absence of the syndrome. After observing a good data flow, all ended up with the following logistic regression model:

$$EED = \alpha + b1*stunting + b2*underweight + b3*wasting + b4*gender + b5*age$$

Where, alfa is the y-intercept, b1 the coefficient of stunting, b2 the coefficient of underweight, b3 the coefficient of wasting, b4 the coefficient of gender and b5 the coefficient of age in months.

From the model, odds ratios and 95% confidence intervals were calculated through exponentiation of coefficients of explanatory variable and results are shown in Table 10 below.

Table 10: Model output for Environmental Enteric Dysfunction

	Odds Value	2.5% CI	97.5% CI	P value
Y-Intercept	3.639602536	0.592847701	26.82623952	0.178177473
Stunting yes	0.464685021	0.12527316	1.505932282	0.220308732
Underweight yes	5.10572021	0.978675366	42.08205887	0.078539597
Wasting yes	6.19E-09	NA	1.62E+103	0.990865822
Gender Male	1.162573583	0.457574566	2.957331044	0.750299182
Age in months	0.977086498	0.935047792	1.019106678	0.285014196

Being of male gender had almost 16% more odds of having EED, and a unit increase in age seemed to lower the odds of EED by about 3%.

(iv) Environmental Enteric Dysfunction Data Simulation

In attempt to increase the statistical power with understanding that, a bigger sample size has less standard error; a sample size of 1000 was simulated from the original 89-sample size. For every variable, a probability of binomial distribution was calculated as per formula below, which was used to obtain mean and standard deviation (SD) of yes cases against no. These values were then simulated to create a table in excel of 1000 sample size with same six (6) variables. The table of simulated values was then attached in R for same univariate, bivariate and multivariate analysis as per tables below.

$$f(x) = \binom{n}{x} p^x (1-p)^{(n-x)} \quad \text{where } x = 0, 1, 2, \dots, n$$

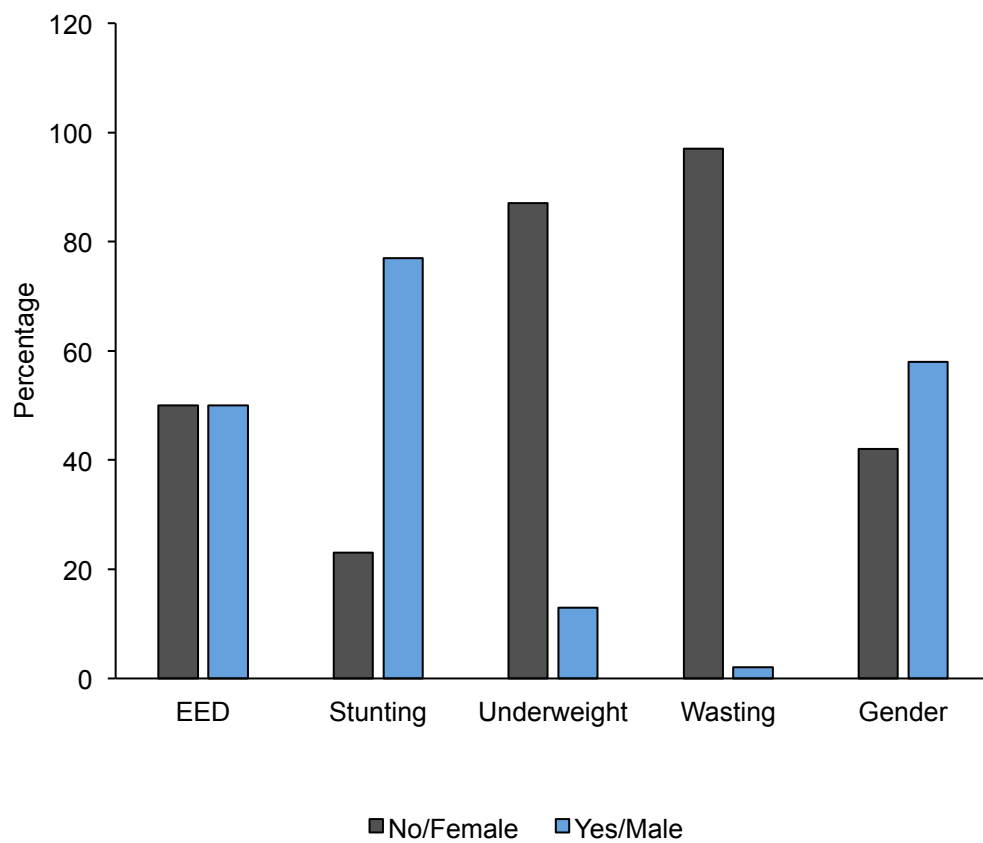


Figure 3: Demographic characteristics of simulated data

Each explanatory variable was cross-tabulated against EED status and then ran chi-square tests.

Table 11: Cross-tabulations and chi-square tests for simulated Environmental Enteric Dysfunction

Variable (factor)	Levels	EED		
		No	Yes	
Stunting	No	122	111	
	Yes	380	387	
	Chi-square results	X-squared = 0.46013	df = 1	p-value = 0.4976
Underweight	No	443	424	
	Yes	59	74	
	Chi-square results	X-squared = 1.8314	df = 1	p-value = 0.176
Wasting	No	485	488	
	Yes	17	10	
	Chi-square results	X-squared = 1.3215	df = 1	p-value = 0.2503
Gender	Male	296	280	
	Female	206	218	
	Chi-square results	X-squared = 0.66001	df = 1	p-value = 0.4166
	Odds Value	2.5% CI	97.5% CI	P value
Y-Intercept	0.9920319	0.8763321	1.122984	0.8993435
Age in months	NA	NA	NA	0.8993435

All independent variables were statistically not significant enough to explain EED. The logistic regression model was formulated using 5 variables also, with EED as outcome variable, and ended up with the following logistic regression model:

$$EED_{sim} = \alpha + b1*stunting_{sim} + b2*underweight_{sim} + b3*wasting_{sim} + b4*gender_{sim} + b5*age_{sim}$$

Where, α is the y-intercept, $b1$ the coefficient of stunting, $b2$ the coefficient of underweight, $b3$ the coefficient of wasting, $b4$ the coefficient of gender and $b5$ the coefficient of age in months. Table 12 below summarizes the odds ratios, their 95% confidence intervals and the p-values.

Table 12: Model output for simulated Environmental Enteric Dysfunction

	Odds Value	2.5% CI	97.5% CI	P value
Y-Intercept	0.956441637	0.709180135	1.289123549	0.769929989
Stunting yes	1.112233088	0.828442636	1.494383594	0.479317611
Underweight yes	1.29991685	0.900394158	1.883881603	0.162916738
Wasting yes	0.572149389	0.249787797	1.243903957	0.16752134
Gender Male	0.893171443	0.694334246	1.148591728	0.378771198
Age in months	NA	NA	NA	0.769929989

4.2 Discussion

4.2.1 Water, Sanitation, Hygiene and Stunting

In recent years beyond 2016, no studies have reported WaSH status in Ruvuma Region despite its high prevalence of stunting (44%). This study has shown that, approximately one in every two children was stunted. Although this study showed no statistical significance with parent or guardian employment status ($p=0.2$, 0.05), it has been reported elsewhere that employed parents/guardians are more likely to be educated and have knowledge of best child's care practices in infection free surrounding (Dotti & Treas, 2016). However, unemployed parents/guardians especially farmers or in business are likely to be less educated, thus associated with poor/limited knowledge on child's care and may reside in poor environment that may harbor potential pathogens (Liu *et al.*, 2016).

Only 4 out of 10 houses treated drinking water, with very few families who used bottled water while most families did not treat water. Since public water is usually treated before distribution (Nishida *et al.*, 2019; Rittmann *et al.*, 1989; Van-Nguyen *et al.*, 2018), this study could safely state for certain that almost all households had access to safe water, although no public tap water was screened for pathogens. From the multivariate analysis, the model showed no statistically significant association between child's stunting and neither water availability and treatment nor availability of latrine facilities: thus, this study found small association between studied WaSH habits and stunting. Humphrey *et al.* (2018) also found no effect of water, sanitation and hygiene interventions on stunting. This lack of association

maybe because of the designs of our study (cross-sectional), so follow-up research designs may provide more solid evidence about the influence of WASH habits on stunting.

Children between birth and 18 months attended clinics the most, with highest number being at 8 months, and the number decreased as the age ascended towards 59 months. The reason for this finding could be that, mandatory vaccination (Chotta *et al.*, 2017; Troeger *et al.*, 2018; USAID.GOV, 2016) for children at birth, 3, 6 and 18 months enabled active clinic attendance by parents/guardians. Also, the stated age range (below 2 years) is when most children get infections frequently (Ceyhan *et al.*, 2018; Hanna-Wakim *et al.*, 2015; Shi *et al.*, 2017), which generally increases the habits for mothers and children to attend hospitals.

Similar to other studies, findings from this study also showed that, most children are stunted during the first 2 years (<24 months) of growth (De Onis & Branca, 2016; Mal-Ed Network Investigators, 2017) but as a child ages, he/she increases a risk for being stunted. Children below 12 months of age are in between the crawling and standing stages, and they get exposed to contaminated environments, which includes putting contaminated objects into their mouths, and thus getting infected easily. Since stunting can result from chronic diarrhea, re-exposure and re-infections, and untreated diarrhea cases at the age of below 24 months (Berhe *et al.*, 2019), put children at high risk for being stunted at later age (>24 months). The increased risk for stunting at a later age may be attributed by the fact that, children above 24 months are no longer breast fed, with less parental care and they are actively playing, especially in less hygienic environments; where they become susceptible to infections such as enteric infections.

This study observed increased risk of stunting being associated with male children. This may be explained by the fact that male children tend to play more actively than female children, hence they get more exposed to dirty environments and pathogens, which may then cause infections like diarrhea, leading to stunting in the end. Interestingly, this finding is logically supported by the fact that, study also indicated that the risk of being stunted can significantly be reduced when the family practices hand washing before food intake, which reduces dirty and contaminated pathogens. The same trend was reported by Torlesse and colleagues that the risk for stunting can be reduced by improved sanitation (toilet), improved water and treatment of drinking water (Dearden *et al.*, 2017; Torlesse *et al.*, 2016). Rah found no association between access to water as a risk for stunting in children and stunting status but reported a

prediction of stunting through access to toilet facility (Rah *et al.*, 2015). Neira and colleagues reported in 2014 the need for more information on solid waste and waste water management (Pruisse *et al.*, 2014), and this current study has found that the municipal solid waste collection system may improve household sanitation significantly. The population in our sample that applied the system was so small but the impact was almost visible. However, it is unfortunate that this municipal system is not distributed throughout Ruvuma (rural and urban), thus most people dig pits for waste disposal. Since fecal disposal by digging pits has been reported to increase the risk of a child to diarrhea (Tumwine *et al.*, 2002), poor garbage disposal methods might be the reason as to why living in resource limited rural Songea puts a child at higher risk for being stunted compared to those residing in urban Songea, due to absence of municipal garbage collection system. Nanan's suggestion for sanitation education as part of intervention in 2003 may be considered for future studies (Nanan *et al.*, 2003). Also, if hand washing were to be practiced at larger scales, risks for stunting would have been lowered significantly. A similar study revealed an effectiveness in improving hygiene by hand washing with soap (Park *et al.*, 2019) while another study indicated an indirect community protection via hand washing before food preparation in a household (Fuller & Eisenberg, 2016). World Health Organization in 2004 had shown reduction in poor hygiene through hand washing and hand washing education by 45% (World Health Organization, 2004).

The main challenge to hand washing is water availability; water source safety and distance, as some use tube well water; which is unsafe and a few walks long distances to get water. Results from this study also revealed that, 6 houses out of 10 had access to clean and safe water, although almost half of the houses don't own tapes or wells within premises; and therefore, water cannot be available at all the time. Additionally, public tape water becomes unreliable and people face water scarcity during the dry season in Songea (September through December), which contributes much to water unavailability and susceptibility of children to infections. Joshi and colleagues (2014) reported that water scarcity in April through June in south Delhi also attributes to limited access to clean water and poor water treatment by households (Joshi *et al.*, 2015).

Moreover, this study found out that diarrhea treatment, toilet sharing, ability of a child to use the toilet and rinsing child's feces into the household's toilet as a disposal method to be significant risk factors to diarrhea disease in children under five years. Since Exley and

colleagues) reported similar sanitation results between private and shared toilets in limited settings and classified both as improved sanitation (Baker *et al.*, 2016; Exley *et al.*, 2015), this study reports improved latrines in all investigated households.

It has been reported that a child's risks to diarrhea may be increased when households share latrines (Crocker & Bartram, 2016), but may be reduced if a child below five years uses the household's toilet directly. Other studies have reported that limited toilet sharing and children who use the toilet had improved sanitation and thus less diarrhea cases (Eisenberg *et al.*, 2007). On the other hand, Tumwine and colleagues found an increased risk to diarrhea if a child's feces is disposed by digging pits (Tumwine *et al.*, 2002), which is supporting our findings of reduced risks with correct rinsing of child's feces in the toilet. If child's feces are disposed into the toilet compared to garbage disposal or leaving it in the open, it keeps the environment hygienic and reduces exposure to pathogens. This keeps a child safe from diarrhea and subsequent EED. The few households (94) that rinsed children's feces into the toilet and yet their children had diarrhea may be caused by poor cleanness of the parent/guardian after rinsing i.e. poor hand wash and perhaps hand washing without soap or running water.

4.2.2 Environmental Enteric Dysfunction

This study has shown higher prevalence of stunting (78%) and EED (57%) in Ruvuma region compared to 44% reported by Tanzania surveillance demographic report (MoH-Z, 2015). The prevalence difference between what was reported in the national surveillance and what is reported in this study may be attributed by the sample sizes involved. National surveillance had a big sample size covering all regions of Tanzania while this part of the study had only 89 children participants from two districts of Ruvuma region, which may not be representative of the national population, as discussed earlier in this document. Also, there was a 5 years' time difference between recorded prevalence from the national survey and this study.

Generally, over half of the cases (stunted children) had confirmed EED syndrome, hence a need for more information on prevalence of diarrhea (and/or other infections) and WaSH services in these children at the study area. Food insecurity may not be the only factor that leads to stunting, as EED syndrome may attribute to increased stunting cases in Ruvuma. Interestingly, less respondents were underweight (height-for-weight) and even very few of

them were wasted (weight-for-age), showing less contribution of food insecurity to stunting as speculated. Similar trend was reported by similar studies (Khan *et al.*, 2019; De-Onis *et al.*, 2019). In contrast to this study, Parpia and colleagues reported no association between food insecurity and low weight of a child at individual household level (Parpia *et al.*, 2020).

EED syndrome was diagnosed to children aged 12 months (1 year) and above, which gave room for EED development and stunting, if presence since the syndrome and stunting take peak at the first 1000 days (24 months) of a child's life; whereas, more than half of all diagnosed children were between the age of 12 to 24 months and more than half of this same group who had EED (Robertson *et al.*, 2019; Lin *et al.*, 2020). Presence of syndrome declined with children at higher age. Equivalently, half number of children from each gender had EED syndrome, with a slight increase in number of male children.

Interestingly from the multivariate analysis, we observed that a stunted child decreased risk for EED syndrome but after increasing the sample size through data simulation, stunting did increase the risk for EED syndrome. The first observation may have been a result of the small sample size, which reduced statistical power, where as a bigger sample size has shown the expected trend of positive association between stunting and EED syndrome. On the same note, EED syndrome is reversible but takes longer up to years while stunting is irreversible.

Child's aging did show a tremendous decrease in the risk for EED, because body immune system and immunity strengthens with getting older in childhood. Since EED is caused by poor WaSH (Gough *et al.*, 2020) and below two years of age is the active playing phase of a child in poor WaSH environment, re-exposures to infections also strengthens the child's immunity. This finding could also, have been contributed by improved hygiene practice by diagnosed children's families.

Lower risk for EED showed by wasting (weight-for-age) factor was probably attributed by the fact that, most wasting cases are treated when they occur as they tend to be acute, as opposed to stunting, which is chronic and always go undiagnosed. Recently, Simon and colleagues have shown positive relationship between wasting and stunting, stating that stunted children are as a result of chronic malnutrition in the form of wasting (Schoenbuchner *et al.*, 2019).

In this study, stunting was not directly associated with family size, co-existing family, parents'/guardians' employment status, source of water, drinking water treatment, distance to

water source, type of toilet, toilet sharing, presence of stagnant waste water within premises, waste water collection service, when a child uses toilet, child's fecal disposal, garbage disposal, schedule for municipal garbage collection system, hand washing, use of soap, child's diarrhea status, and parents' disease status. Also, diarrhea among children was not associated with gender, stunting, hospital, location, parents'/guardians' employment status, source of water, drinking water treatment, distance to water source, type of household's toilet, presence of stagnant waste water within premises, waste water collection service, garbage disposal, schedule for municipal garbage collection system, hand washing, use of soap, and parents' disease status.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This research aimed at determining the prevalence of stunting using hospital-based cross-sectional study and assessing EED in children using a case-control study in the Ruvuma Region of Tanzania. The findings revealed that stunting was associated with gender, age in months, and hand washing before having food while the case-control study showed no linkage between EED and stunting. Diarrhea disease was associated with ability of a child to use the household's toilet and toilet sharing among households. Most of these correlates can be modified through public health interventions that target undernutrition. Moreover, a longitudinal population-based study in Ruvuma and other regions with high prevalence of stunting in Tanzania is warranted, to comprehensively assess determinants of stunting and their association with EED, food habits, WaSH services and infections in children below the age of 5 years. Environmental Enteric Dysfunction (EED) is reversible but its recovery takes a very long time, depending on the amount of exposure. Early detection of EED in infants is therefore essential for prevention of its adverse effects, including stunting. Recent studies have shown that treatment of EED at an individual level and interventions, such as improved WaSH services, have reduced EED and stunting to a minimal effect. New intervention approaches are still needed if we are to liberate the problem in Tanzania.

5.2 Recommendations

This study recommends combined interventions that address stunting and EED through WaSH campaigns, food security, promotion of exclusive breastfeeding in the first six months of a child's life, complementary feeding, nutrients and vitamin supplementation during gestation, and anti-inflammatories prescription to children.

This study also recommends future research on assessment of EED in children less than two years to target the window period. The investigation of EED should also include more than one biomarker since L:M ratio test indicates intestinal permeability only. Other causal factors should also be included in this investigation, including WaSH services and presence/absence

of diarrhea or/and other infections. The study should follow up and monitor participants for a period of not less than six to twelve months with a bigger sample size to minimize error.

This study further recommends future studies to incorporate laboratory screening of tap and well water for enter-pathogens in order to say for sure if the water is safe or not. Also, studies on WaSH should consider collecting information on child feeding habits, including breastfeeding and complimentary feeding, as it may be one of the factors for diarrhea and hence stunting in breastfeeding children.

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APPENDICES

Appendix 1: Questionnaire

QUESTIONNAIRE FOR Water, Sanitation and Hygiene

Patient Information

Name:

Address:

Date of Birth: (Day) (Month) (Year)

Age:

Gender: Male ☐ Female ☐

Height (cm):

Weight (Kg):

Family Information

Name:

Contact details:

Total number of family member:

Total children < 5 years:

Another family hosted in the house: Yes/No. Number of guests:

Parents Employment details:

Father (male guardian): Employed ☐ Unemployed ☐

If Unemployed: Farmer ☐ Businessman ☐ others ☐

Mother (female guardian): Employed ☐ Unemployed ☐

If Unemployed: Farmer ☐ Businessman ☐ others ☐

Household information

Location:

Type of the house:

1. What is the main source of drinking-water for members of your household?
 - a. Public tap/standpipe
 - b. Tube well/borehole
 - c. Unprotected spring
 - d. Surface water (river, dam, lake, pond, stream, canal, irrigation channels)
 - e. Other (specify)
2. What is the main source of water used by your household for other purposes, such as cooking and hand washing?
 - a. Public tap/standpipe
 - b. Tube well/borehole
 - c. Unprotected spring
 - d. Surface water (river, dam, lake, pond, stream, canal, irrigation channels)
 - e. Other (specify)
3. How long does it take to go there, get water, and come back?
 - a. No. of minutes
 - b. Water on premises
 - c. DK
4. Do you treat your water in any way to make it safer to drink?
 - a. Yes
 - b. No
 - c. DK
5. How do you treat water to make it safe before drinking?
 - a. Boil
 - b. Add bleach/chlorine
 - c. Strain it through a cloth
 - d. Use a water filter (ceramic, sand, composite, etc.)
 - e. Let it stand and settle
 - f. Other (specify)

- g. Don't Know
- 6. What kind of toilet facility do members of your household usually use?
 - a. Flush toilet
 - b. Pit latrine
 - c. Unknown place/not sure/DK where
- 7. Do you share this facility with other households?
 - a. Yes (go to 8)
 - b. No
- 8. How many households use this toilet facility?
 - a. How many other households share this toilet?
 - b. Can any member of the public use this toilet? (Yes/No)
 - c. DK
- 9. Do you have stagnant or sewage water near your house?
 - a. Yes
 - b. No
 - c. DK
- 10. Are you served by waste-water network?
 - a. Yes
 - b. No
 - c. DK
- 11. Do your children (< 5 years) use toilet?
 - a. Yes
 - b. No
 - c. DK
- 12. How do you dispose [name of youngest child]'s stool/feces?
 - a. Child use toilet/latrine
 - b. Put/rinsed into toilet or latrine
 - c. Put/rinsed into drain or ditch
 - d. Thrown into garbage
 - e. Buried
 - f. Left in the open
 - g. Other (specify)
- 13. How do you get rid of the solid waste from your household?

- a. Dig a pit
 - b. Burn it
 - c. Use the municipal collection system (go to 13)
14. How often does a municipal system collect solid waste?
- a. Once a week
 - b. Once in two weeks
 - c. Once a month
 - d. Others (specify)
15. When do you usually wash your hands?
- a. After using the toilet
 - b. Before meal time
 - c. After meal time
 - d. Before bed
 - e. Every after house chores
 - f. Before cooking
 - g. Others
16. Do you use soap when washing your hands?
- a. Yes
 - b. No
17. Has any child < 5 years old or member of the family in the household recently had diarrhea?
- a. Yes (go to 18)
 - b. No
 - c. DK
18. Was it treated?
- a. Yes
 - b. No
 - c. DK
19. How often do your children get diarrhea?
- a. Once per week
 - b. Once per month
 - c. Twice per month
 - d. DK

e. Others

20. Do you have any sick child in the family?

a. Yes

b. No

If yes, what kind of sickness?

21. Do you have any illness among the parents/guardians?

a. Yes

b. No

If Yes, Please name it

Appendix 2: Student Introduction letter from NM-AIST

THE NELSON MANDELA
AFRICAN INSTITUTION OF SCIENCE AND TECHNOLOGY
(NM-AIST)

School of Life Sciences and Bioengineering

Direct Line: +255 272555070
Mobile Phone: +255272970005
Fax: +255 272555071
E-mail: deanlsbe@nm-aist.ac.tz



Tengeru
P.O. Box 447
Arusha, TANZANIA
Website: www.nm-aist.ac.tz

Ref. No. NM-AIST/M.501/T.17

Date: 29th November, 2018

To whom it may concern,

RE: INTRODUCTION OF GRANTINA MODERN

This is to introduce that, Miss. Grantina Modern is a master student at Nelson Mandela African Institution of Science and Technology under the school of Life Science and Bioengineering (LiSBE).

Her study will focus on assessment of environmental enteric dysfunction (EED) in healthy and undernourished children-a crosstalk between EED and stunting. The study is aiming at assessing the link between EED and stunting in children, in order to possibly suggest new nutritional interventions that may effectively work when dealing with stunting.

Kindly, any assistance from your office will be highly appreciated to accomplish her mission.

Sincerely,

Nelson Mandela African Institution
of Science and Technology
(NM AIST - ARUSHA)
P. O. Box 447
Tel: +255 27 2555071

Dr. Gabriel M. Shirima
Dean School of Life Science and Bio-engineering

Academic for Society and Industry

Appendix 3: Ethical Clearance



Kibong'oto Infectious Diseases Hospital- Nelson Mandela African Institution of Science and Technology- Centre for Educational Development in Health, Arusha (KIDH-NM-AIST-CEDHA) -KNCHREC

RESEARCH ETHICAL CLEARANCE CERTIFICATE

Research Proposal No: KNCHREC0011

14TH MARCH 2019

Study Title: Assessment of Environmental enteric dysfunction (EED) in healthy and undernourished children-a crosstalk between EED and Stunting

Study Area: THE NELSON MANDELA AFRICAN INSTITUTION OF SCIENCE AND TECHNOLOGY

PI Name: GRANTINA MODERN

Co-Investigator:

Institutions: School of Life Science and Bio-Engineering (LiSBE) of the Nelson Mandela African Institution of Science and Technology

The Proposal has been approved by KNCHREC on 14th March 2019

1. Subject to this approval you will be required to submit your progress report to the KNCHREC, National Institute of Research and Ministry of Health Community Development Gender Elderly and Children
2. Publication of your findings is subject to presentation to the KNCHREC and NIMR Approval.
3. Copies of final publication should be made available to KNCHREC, National Institute of Research and Ministry of Health Community Development Gender Elderly and Children

Duration of Study Renewal: Subject to Renewal within ONE YEAR

Span From: 14th March 2019 to 13TH March 2020.

.....
Mr. Simon Njeya
Secretary
KNCHREC


Chairperson
KNCHREC

Appendix 4: RMO - Ruvuma Research clearance

THE UNITED REPUBLIC OF TANZANIA
PRESIDENT'S OFFICE
REGIONAL ADMINISTRATION AND LOCAL GOVERNMENT

RUVUMA REGION

Tel. Nos. 025-2602256/2602238

Fax No. 2602144

E-mail. ras.ruvuma@tamisemi.go.tz

Tovuti: www.ruvuma.go.tz



Regional Commissioner's Office,
P.o. Box 74,
SONGEA.

Ref. No. AFY/R.11/VOL. VIII/7

27th March, 2019.

Medical Officer Incharge,
Songea Regional Referral Hospital,
P. O. Box 5,
SONGEA.

Medical Officer Incharge,
Peramiho Mission Hospital,
P. O. Box 19,
SONGEA


RE: INTRODUCTION TO M/S. GRANTINA MODERN

Reference is made to the above subject.

The above named person is a second year student from the Nelson Mandela African Institution of Science and Technology. The student has been permitted to conduct a research on Assessment of Environmental enteric dysfunction (EED) in healthy and undernourished children – a cross talk between EED and Stunting in Songea Regional Referral Hospital and Peramiho Mission Hospital. All necessary requirements have been observed to legalize this research.

Based on the above explanation am requesting you to allow the above named person to conduct the stated research in your respective hospital.

With regards,


Dr. Jairy N. Khanga
REGIONAL MEDICAL OFFICER
RUVUMA

Copy:- Grantina Modern

RESEARCH OUTPUTS

Journal papers

Published research article

Modern, G., Sauli, E., & Mpolya, E. (2020). Correlates of diarrhea and stunting among under-five children in Ruvuma, Tanzania; a hospital-based cross-sectional study. *Scientific African*, 8(1), 1-9. <https://doi.org/10.1016/j.sciaf.2020.e00430>.

Poster presentations

Assessment of Environmental Enteric Dysfunction in healthy and undernourished children: a crosstalk between EED and Stunting