ENVIRONMENTAL HYGIENE, FOOD SAFETY AND GROWTH IN LESS THAN FIVE YEAR OLD CHILDREN IN ZIMBABWE AND ETHIOPIA

A Dissertation

Presented to the Faculty of the Graduate School

of Cornell University

In Partial Fulfillment of the Requirements of the Degree of

Doctor of Philosophy

by

Francis Muigai Ngure

August 2012

ENVIRONMENTAL HYGIENE, FOOD SAFETY AND GROWTH IN LESS THAN FIVE YEAR OLD CHILDREN IN ZIMBABWE AND ETHIOPIA

Francis Muigai Ngure, Ph.D.

Cornell University 2012

Childhood stunting remains a significant public health challenge with adverse developmental and health outcomes in life and over generations. Efficacious dietary interventions have not achieved full linear growth in height deficient children. This dissertation explores novel causes of poor growth in young children by: identifying pathways of fecal-oral transmission of fecal bacteria in rural Zimbabwean infants using in-depth observation methods (chapter 2), examining the role of poor water, sanitation and hygiene in predicting stunting, in the context of infant and young child feeding, in Ethiopia (Chapter 3) and assessing the extent of aflatoxin exposure in Zimbabwean women and its association with stunting in young children (Chapter 4).

In chapter 2, soil and chicken feces ingestion were identified as key pathways for fecal-oral transmission of bacteria in rural Zimbabwean infants. In Chapter 3, poor environmental hygiene was associated with linear growth faltering, independent of socio-economic status, infant feeding and recent morbidity, in Ethiopian children 24-59 months of age. In chapter 4, significant aflatoxin exposure was associated with severe stunting in a dose response manner in rural Zimbabwean children 6 to 59 months of age.

The combined results from the three research projects identified environmental hygiene and aflatoxin exposure as two novel causes of stunting in African infants. Existing water, hygiene and sanitation interventions are not sufficient to protect infants

and young children from fecal bacteria ingestion through soil and poultry feces. In addition, existing hygiene interventions do not directly address improving household environmental hygiene. Designing effective hygiene interventions will require in-depth understanding of the context and how caregivers and infants interact with their environment. Effective aflatoxin exposure control might also be critical in achieving full growth potential in young children. Our findings raise the need for low cost strategies for aflatoxin control and a holistic approach in designing context- and age-specific hygiene interventions to prevent childhood stunting.

BIOGRAPHICAL SKETCH

Francis Muigai Ngure was born and grew up in Elburgon, Nakuru County, Rift Valley Province, Kenya. He studied in local public schools and later joined Egerton University in the neighboring Njoro division. While on school holidays, he was actively involved in *Shamba* system farming; a mixed farming system initiated by the Kenyan government to promote reforestation in the Mau Forest, in Elburgon. During these early years that he was exposed to the food system in Kenya, long before the dream of studying nutrition had been envisaged. In these formative years in farming and hard work at school he learned discipline, diligence and self-drive. He is greatly indebted to his parents for cultivating these virtues and the quest for academic excellence.

After studying for Bachelor of Science and Master of Science degrees in Food Science in Egerton University, Njoro, Nakuru County, Francis moved to Nairobi and worked with a Norwegian Food Company, Compact AS, specializing in processing ready-to-eat supplementary and therapeutic (RUSFs and RUTFs) food for nutrition in emergency settings. He was primarily involved in local consultancy and technology transfer to local partners like Embu Food Industry. It is during research to develop new food products and marketing Compact AS therapeutic foods to humanitarian organizations that his dream to study nutrition was born.

Having experienced the challenges facing the food system in Kenya in his early life, and with experience in the food industry, he joined Division of Nutritional Sciences, Cornell University, with a keen interest in understanding the role of

micronutrients (in such RUTFs as he marketed) in improving health and growth outcomes in children. In addition, Francis was interested in novel, affordable and sustainable ways to alleviate food and nutritional insecurity, which the costly RUTFs could not sustain.

Cornell provided him with a motivating and excellent interdisciplinary environment for learning and personal growth. His academic and research work was supervised by Rebecca Stoltzfus. After completing his dissertation, Francis will conduct post-doctoral research on understanding the interaction of WASH (water, sanitation and hygiene) and infant feeding practices in predicting stunting in other contexts. Later, he will continue with nutrition research in Kenya, before taking his experience to the university to teach and mentor students.

To God, with whom all things are possible (Matthew 19:26b).

May this work bring praises and glory to your name.

To my mom and dad, you are the best and you gave me the best.

To Mary, your love and support is invaluable, thank you.

To Patrick, Peter, George, Moses and Anthony,

I treasure your support and friendship.

ACKNOWLEDGEMENTS

Special committee

I thank Rebecca Stoltzfus, the chair of my special committee, my academic advisor and mentor for invaluable time and efforts towards my studies, research and personal growth. Your mentorship motivated me to work harder and offer the best to students whom I will interact with in future. I also thank other members of my special committee: Kathryn Boor, Patricia Cassano and Robert Parker, for guiding through my various aspects of my research and studies at Cornell. Your insights and advice enriched my learning greatly.

Funding

My studies and research would have not been possible without the generous support of Cornell University, Department for International Development (DFID), United Kingdom, Borlaug Leadership Enhancement in Agriculture Program (LEAP), Division of Nutritional Sciences Small Grants and First Presbyterian Church, Ithaca. Thank you for the financial support.

Our collaborators

Special thanks to Jean Humphrey and Mduduzi Mbuya for guidance on my research in Zimbabwe, and all Zvitambo research staff for their support in conducting the Infant Observation and Aflatoxin studies. This work would have not been possible without your support. I thank the infants' observation team; Florence Majo, Rukundo

and Dadirai, Microbiology Team; Kuda, Sandra, Margaret and Exevia. My stay in Zvitambo was a great learning experience and you all made it memorable.

I thank Purnima Menon of IFPRI, New Delhi for allowing me to work on the Alive and Thrive *initiative* baseline data analyses. Special thanks to Purnima, Disha Ali, Rahul Rawal and Michael Tedla for a fruitful collaboration. This collaboration provided a rare opportunity to work with a huge data set to answer pertinent research questions. Your insights, thoughts and advice are greatly acknowledged.

Research groups

Thanks to Sera Young for the insights in our discussions on infant geophagia and other aspects of my research. I thank all my colleagues in Stoltzfus and Young research group. I also thank all colleagues and members of Cornell faculty in Maternal and child nutrition research group, led by Kathryn Rasmussen, for great feedback into my research.

Cornell Statistical Consulting Unit

I must gratefully acknowledge Francoise Vermeylen, Haim Bar and Jing Yang for advice and statistical support on data analyses. Thank you for your time and effort.

Friends

Special thanks to Amos and Jackie Kung'u who helped me settle down in Ithaca during my first winter experience (January, 2008). Jackie, your advice in my initial stages of my program carried me through subsequent years and saved me time. Amos,

your friendship was a great encouragement during a challenging transition. Taji, I enjoyed your company any time.

Thanks to Ruxy, Cynthia, Tayo and Joseph. You have been wonderful colleagues and friends. I am happy to get to know you and share wonderful learning experiences in Cornell.

Special thanks to my best friend in Ithaca, Peter Lundquist. Our time together is memorable and you were a great motivation during the writing. Thank you for the prayers. I enjoyed my stay with you and Tiju and your cheered me on.

To all my CICF friends, Bethel Grove Church and East Africans at Cornell, thank for your prayers and moral support.

Family

To my mom and dad, you have always been there for us as a family and I thank you for all the support. To my siblings: Patrick, Peter, George, Moses, and Anthony, I thank you for believing in me. Mary, you have been a great source of encouragement and thank you for incredible sacrifices to be with me in Zimbabwe, Ethiopia and Ithaca. You are the very best.

TABLE OF CONTENTS

BIOGRAPHICAL SKETCH iii
DEDICATION v
ACKNOWLEDGEMENTS vi
LIST OF FIGURES xii
LIST OF TABLES xiii
CHAPTER 1 1
INTRODUCTION 1
Childhood malnutrition 1
Water, sanitation and hygiene and child growth 2
Environmental enteropathy and linear growth 4
The underlying hypothesis 5
Research questions 8
REFERENCES 10
CHAPTER 2 14
FORMATIVE RESEARCH ON HYGIENE BEHAVIOURS AND GEOPHAGY AS PART OF INTERVENTIONS TO IMPROVE INFANTS' GROWTH IN ZIMBABWE 14
ABSTRACT 14
INTRODUCTION 15
Study Site and Sample Frame 17
Ethics 17
Caregiver-Infant Observation 18
Microbiological analyses (May--June 2011) 19
Data Analysis 21
RESULTS 21
Household Characteristics 21
General Hygiene 22
Hand washing and nappy change practices 22
Frequency of infant mouth contact of potential fecal-oral vectors 23
Bacterial contamination of the key vectors 31
DISCUSSION 36
CONCLUSION 41

REFERENCES 44
CHAPTER 3 47
PREDICTORS OF POOR NUTRITIONAL STATUS IN LESS THAN 5 YEAR OLD ETHIOPIAN CHILDREN- AN EMPHASIS ON WATER, SANITATION AND HYGIENE (WASH) 47
ABSTRACT 47
INTRODUCTION 48
Research Questions 50
SUBJECTS AND METHODS 51
Alive and Thrive Initiative Baseline Data 51
Sampling of households and respondents 52
Household questionnaire 53
Data Analysis 54
RESULTS 56
Maternal, child and household characteristics 56
Infant feeding in Tigray and SNNPR 61
The prevalence of growth faltering in Tigray and SNNPR 64
Significant predictors of HAZ and WHZ for 0-5.9 months old 66
Significant predictors of HAZ and WHZ for 6-11.9 months old 70
Significant predictors of HAZ and WHZ for 12-23.9 months old 73
Significant predictors of HAZ and WHZ for 24-59.9 months old 76
DISCUSSION 87
The association of WASH and nutritional outcomes 87
Association between IYCF indicators and nutritional outcomes 90
CONCLUSION 92
APPENDIX 94
REFERENCES 101
CHAPTER 4 105
AFLATOXIN EXPOSURE AMONG MOTHERS IS ASSOCIATED WITH SEVERE STUNTING IN 6-59 MONTH OLD CHILDREN IN ZIMBABWE 105
ABSTRACT 105
INTRODUCTION 106
Study Population and Sampling Frame 107
METHODS 108

Data Analysis 109
RESULTS 109
DISCUSSION 114
REFERENCES 120
CHAPTER 5 124
ENVIRONMENTAL EXPOSURES AND IMPLICATIONS FOR CHILD HEALTH AND GROWTH 124
Conclusions and recommendations for research 130
REFERENCES 133

LIST OF FIGURES

Figure 1.1: The underlying hypothesis- adapted and modified from Humphrey (19)..... 7

Figure 3.1: The conceptual framework..... 51

Figure 3.2: Linear growth faltering by age of all children...……..65

Figure 3.3: Prevalence of stunting and wasting in Tigray and SNNPR..... 66

Figure 3.4: Association between water and sanitation variables and Height-for-age Z scores (HAZ) for all 24-59 months old 79

Figure 3.5: Association between hygiene index and Height-for-age Z scores (HAZ) for all 24-59 months old..... 79

Figure 3.6: Association between water and sanitation variables and weight-for-height Z scores (WHZ) for all 24-59 months old 80

Figure 3.7: Association between hygiene index and weight-for-height Z scores (WHZ) for all 24-59 months old 80

Figure 4.1: Odds of severe stunting with exposure category (6-59 months old)..... 112

Figure 4.2: Percent of exposed women by province 112

Figure 4.3: Aflatoxin B1 exposure distribution by region for all women..... 113

Figure 5.1: Rural Ethiopia- SNNPR..... 128

Figure 5.2: Rural Zimbabwe..... 129

LIST OF TABLES

Table 2.1: Maternal and household characteristics of 23 mother-infant dyads in rural Zimbabwe 25

Table 2.2: General hygiene characteristics of infant's environment during infant observation 27

Table 2.3: Mother's hand washing practices, number of events (%)..... 28

Table 2.4: Infants' hand washing practices 30

Table 2.5: Key potential vectors for feco-oral transmission of bacteria..... 30

Table 2.6: Overall mean and number of samples (%) in each category of *E. coli* counts 32

Table 2.7: Overall mean and number of samples (%) in each category of other bacteria counts..... 33

Table 2.8: Infant feces (nappy wash waste water) disposal..... 36

Table 3.1: Scores for IYCF indicators for each age group 55

Table 3.2: Child, maternal and household characteristics in two regions in Ethiopia, June 2010..... 58

Table 3.3: Infant and young child feeding indicators by age group and region 62

Table 3.4: Regression coefficients of the WASH[1], IYCF[2] and Full[3] Models (0-5.9 months) 68

Table 3.5: Regression coefficients of the WASH[1], IYCF[2] and Full[3] models (6-11.9 months old)..... 71

Table 3.6: Regression coefficients of the WASH[1], IYCF[2] and Full[3] models (12-23.9 months old)..... 74

Table 3.7: Regression coefficients of the WASH[1], IYCF[2] and Full[3] Models (24-59.9 months) 77

Table 3.8: Adjusted means of HAZ and WHZ for 0-5.9 months old infants 81

Table 3.9: Adjusted means of HAZ and WHZ for 6-23.9 months old children 83

Table 3.10: Adjusted means of HAZ and WHZ for 24-59.9 months old children..... 85

Table 4.1: Maternal and child characteristics by aflatoxin exposure (n =199)..... 110

Table 4.2: Percent stunted and severely stunted by age category (n=199)..... 110

Table 4.3: The association between aflatoxin exposure and stunting or severe stunting in 6-59 months old..... 111

CHAPTER 1

INTRODUCTION

Childhood malnutrition

Stunting (low height-for-age) and wasting (low weight-for-height) are two conditions and indicators of childhood malnutrition. Stunting is a measure of chronic malnutrition, while wasting is a measure of more transient or short-term, acute malnutrition. Stunting is a major risk factor for diminished survival and child mortality. Stunting is irreversible beyond 3 years of age (1) and has long term consequences, which include short adult height, reduced intellectual development and economic productivity in later life (2). The causes of malnutrition are many and complex. The main causes are intrauterine growth restriction, inadequate nutrition to support rapid growth and development of infants, and frequent infections in early life (3). The causes for child undernutrition have previously been classified as immediate, underlying and basic in the UNICEF framework of the causes of maternal and child undernutrition (4). The immediate causes, inadequate nutrition and disease, are influenced by underlying causes of household food security, child care practices and unhealthy household environment and lack of health care services. The underlying causes are influenced by environmental, economic and sociopolitical contextual factors including income poverty.

Existing programmatic interventions designed to improve nutrition and prevent related diseases can reduce stunting at 36 months by 36%, as previously reported in the Maternal and Child Undernutrition Lancet Series (1). Rigorous diet interventions have achieved at best 0.7 z score improvement (5), one third of the average linear growth

deficit (-2.1 HAZ) seen for Asian and African children within the first year of life. Additional diet supplementation studies and infant feeding behavior change interventions may not achieve more benefit in linear growth, above that which has already been reported.

A majority of the 178 million under 5 year old children who are stunted live in Sub-Saharan Africa and south-central Asia (4). With 40% under 5 year old children stunted in Africa, tackling childhood stunting is a high priority for reducing the burden of disease, child mortality and fostering economic development in Africa. The national prevalence of stunting in less than 5 year old children remains high, 44% in Ethiopia (6) and 32% in Zimbabwe (7), the two nations where this research was conducted. While progress has been made in reduction of childhood stunting in Ethiopia (14% reduction between 1999 and 2011), little progress has been made in Zimbabwe; only a 2% reduction between 2000 and 2011 (6, 7). The overall goal of this dissertation is to advance the prevention of childhood stunting by providing a greater understanding of risk factors for stunting in resource poor contexts.

Water, sanitation and hygiene and child growth

Work productivity of rural people is not only dependent on available farming resources, markets, education, and food and nutrition security but also on their health status. Unhealthy farm laborers incur loss in working hours and lower productivity. The labor intensive agricultural sector suffers when the rural people have to take care of the sick or take time to recover from ill health. Health shocks and health care costs from disease burden at household level exacerbate income poverty necessary for food production, and consequently limit coping ability for the rural livelihoods (8, 9).

A huge disease burden is associated with poor water, hygiene and sanitation conditions and is largely preventable with proven cost-effective interventions (10). Poor water, sanitation and hygiene are responsible for about 50% of maternal and childhood underweight, primarily through the well-described synergy between diarrheal diseases and undernutrition, whereby one increases vulnerability to the other (2, 11, 12). Diarrhea alone kills more young children each year than HIV/AIDs, tuberculosis and Malaria combined (13), and key to diarrhea control are water, sanitation and hygiene (WASH) interventions. Hygiene promotion to prevent diarrhea is the most cost effective health intervention in the world, at only $3.35 per Disability-adjusted Life Year (DALY) loss averted, followed by sanitation promotion at $11.15 per DALY loss averted (14). Globally, prevention of sanitation and water related diseases could save $7 billion per year in health care system costs and an additional $3.6 billion per year from the value of deaths averted (9).

Despite the efforts to provide clean water and hygiene education, the burden from respiratory and gastrointestinal diseases associated with poor growth of infants remains high. The role of water and sanitation has been studied especially in regard to diarrhea as the outcome. However, the relative contribution of diarrhea to stunting remains unresolved. The Lancet Maternal and Child Nutrition Series estimated that hygiene and sanitation interventions implemented with 99% coverage would reduce diarrhea incidence by 30%, which would in turn reduce the prevalence of stunting by only 2.4% at 36 months of age (1).

Studies of improved water supply and sanitation have shown benefits to weight gain and linear growth. Using nationally representative sample from eight countries,

Esrey (15) estimated improvement in sanitation was associated with 0.07 to 0.46 increments in weight-for-age Z score (WAZ) and 0.06 to 0.65 increments in Height-for-age Z score (HAZ). Improvement in water supplies was associated with 0.07 to 0.139 increments in WAZ and 0.01 to 0.38 increments in HAZ. At 2 years of age Peruvian children with the worst conditions for water source, water storage and sanitation were 1.0 cm shorter than children with the best conditions (16). Infants from families with latrines and increased water usage gained 1.1 kg and 2.1 cm more than those from families with increased water usage only (16). However, such studies have not studied the effect of environmental hygiene. In studies looking at growth outcomes, improvement of water supply has been associated with gain in length, but the magnitude of that effect has been less than that of improved sanitation. Evidence of a complementary role of the two has not been conclusive (15, 17, 18).

Environmental enteropathy and linear growth

In the Gambia, dietary inadequacy and diarrhea were not associated with growth faltering, but urinary lactulose to mannitol ratio - an indicator of intestinal permeability- explained 39% of ponderal and 43% of linear growth failure (19). A subsequent study by the same investigators demonstrated that translocation of immunogenic macromolecules across a permeable gut leads to stimulation of the systemic immune processes and subsequent growth impairment (20). They reported elevated levels of total IgG and IgG-endotoxin- core antibody (EndoCAb), and abnormal intestinal permeability as measured by lactulose to mannitol (L/M) ratio (20). IgG, EndoCAb and L/M ratio were negatively correlated with each other and negatively correlated with linear and ponderal growth. EndoCAb indicates an immune response to endotoxin, a

component of gram negative bacteria cell wall probably derived from fecal contamination (21).

Humphrey (21) has recently hypothesized that exposure to larger quantities of fecal bacteria due to poor sanitation and hygiene is the cause of environmental enteropathy. She furthermore hypothesized that the primary causal pathway from poor sanitation and hygiene to stunting is environmental enteropathy (EE) and not diarrhea. EE is an energy intensive subclinical condition, characterized by villous atrophy, crypt hyperplasia, increased permeability, inflammatory cell infiltrate and modest malabsorption (22). These processes impair absorptive and barrier functions of the small intestine mucosa lining.

Several studies have shown an association between aflatoxin exposure and poor growth (23, 24, 25) and numerous animal experiments have demonstrated the same (26). A recent review suggests biologically plausible mechanisms by which exposure to mycotoxins affect growth (27) primarily by mediating intestinal damage through: 1) inhibition of protein synthesis (Aflatoxin and Deoxynivaenol); 2) an increase in systemic pro inflammatory cytokines (Deoxynivaenol); and 3) inhibition of ceramide synthase (Fumonisin). Subsequent downstream pathways to impaired growth are remarkably similar to the proposed causal pathways for EE (27).

The underlying hypothesis

This dissertation examines the proposed causes of EE as conceptually represented in the underlying hypothesis, **Figure 1.1**. Exposure to fecal bacteria from poor WASH and aflatoxin during complementary feeding can synergistically or independently initiate EE. Subsequently the energy intensive immune stimulation will

lead to growth faltering. Recurrent infections (e.g. diarrhea) in early life can cause malnutrition, which increases susceptibility to infection and hence a vicious cycle. Both infections and malnutrition contribute directly to child mortality.

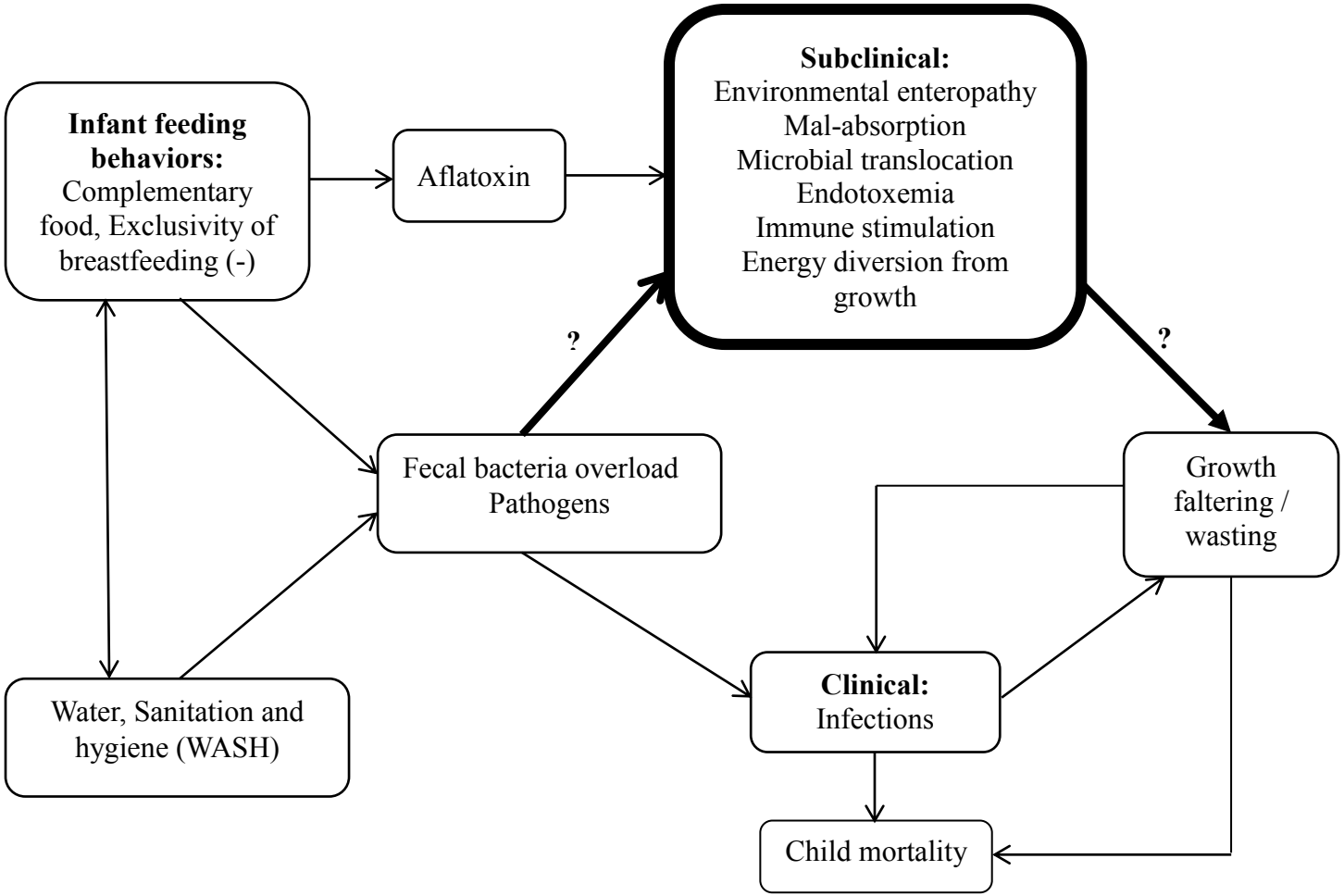

Figure 1.1: The underlying hypothesis- adapted and modified from Humphrey (19)

Research questions

In light of this background, this dissertation addresses the following objectives 1) identify the key fecal oral vectors (objects that are put into with babies' mouths) for infants and quantify the microbiological contamination of the most common vectors among selected households in rural Zimbabwe; 2) determine the prevailing status of Water Sanitation and Hygiene (WASH) and Infant and Young Child Feeding (IYCF) practices in Ethiopia and the relationship between WASH and IYCF in predicting stunting and wasting; 3) determine the extent of aflatoxin exposure and its association with stunting in rural Zimbabwe.

Dietary interventions have been able to solve only one third of the world's problem of chronic under nutrition (stunting) in children. An in-depth understanding of other non-dietary causes is therefore needed to complement the role of diet. The first paper (Chapter 2) is a study conducted in rural Zimbabwe using in-depth observation methods. Twenty-three mother-infant dyads were observed for 6 hours for WASH related behaviors, and especially to identify key fecal-oral vectors (objects that are put into with babies' mouths) and to assess their microbiological quality. The objective of the study was to determine the key routes of fecal-oral transmission of pathogens, hypothesized to be important in the etiology of environmental enteropathy. Identifying key vectors is a critical step to develop an education intervention of hygiene and sanitation to reduce the burden of overt and covert disease conditions that contribute greatly to poor growth in low income countries like Zimbabwe.

The second paper (Chapter 3) explores the association of WASH and poor nutrition outcomes among under 5 year old children in rural Ethiopia and whether the

effects of WASH are independent of IYCF. With the growing need to incorporate IYCF strategies into packages of health (e.g. WASH), nutrition and agriculture, this chapter provides a broader understanding of how the two clusters of practices predict poor nutrition outcomes using multivariate analyses on a cross-sectional data of Alive and Thrive initiative baseline data, 2010.

The third paper (Chapter 4) explores the extent of aflatoxin exposure in mothers with under 5 year old children and its association with stunting in Zimbabwe. Aflatoxin exposure is strongly associated with stunting in dose response manner in West Africa (21). Corn and peanuts are commonly consumed in rural Zimbabwe and they are a good milieu for the growth of aflatoxin producing molds. Low income countries like Zimbabwe lack in food safety screening technology. We hypothesized exposure to high levels of aflatoxins might be common and might further exacerbate the burden of malnutrition.

REFERENCES

1. Bhutta AZ, Ahmed T, Black RE, Cousens S, Dewey K, Giugliani E, Haider BA, Kirkwood B, Morris SS, Sachdev HPS, Shekar M, for the Maternal and Child Undernutrition Study Group (2008). What works? Interventions for maternal and child undernutrition and survival, *Lancet,* DOI:10.1016/S0140-6736(07)61693-6
2. Victora CG, Adair L, Fall C (2008). Maternal and child undernutrition: consequences for adult health and human capital. *Lancet* 371: 340–57.
3. Frongillo EA, Jr. (1999) Symposium: Causes and Etiology of Stunting. Introduction. *J Nutr;*129 (2S Suppl):529S-30S.
4. Black RE, Allen LH, Bhutta ZA, Caulfield LE,Onis M, Ezzati M, Mathers C, RiveraJ, (2008). Maternal and child undernutrition: global and regional exposures and health consequences. Lancet 371: 243- 260.
5. Dewey KG, Adu-Afarwuah S (2008). Systematic review of the efficacy and effectiveness of complementary feeding interventions in developing countries. *Maternal and Child Nutrition;* 4(S1):24–85.
6. Central Statistical Agency [Ethiopia] and ICF International (2012). Ethiopia Demographic and Health Survey 2011. Addis Ababa, Ethiopia and Calverton, Maryland, USA: Central Statistical Agency and ICF International.
7. Zimbabwe National Statistics Agency (ZIMSTAT) and ICF International (2012). Zimbabwe Demographic and Health Survey 2010-11. Calverton, Maryland: ZIMSTAT and ICF International Inc.

8. Pinstrup-Andersen P, *Ed.* (2010). The African food system and its interaction with human health and nutrition. *Cornell University Press*.
9. Cairncross S, Valdmanis V (2006). Water supply, sanitation, and hygiene promotion. In: Jamison DT, Breman JG, Measham AR, et al. (2006) Disease control priorities in developing countries, *2nd ed*. New York: Oxford University Press. pp 771–792.
10. Bartram J, Cairncross S, (2010). Hygiene, sanitation, and water: forgotten foundations of health. *PLoS Medicine www.plosmedicine.org*, 7:11,e1000367
11. World Bank (2008) Environmental health and child survival: epidemiology, economics, experience. Washington, DC: World Bank. 135 p.
12. Blossner M, de Onis M (2005). Malnutrition: quantifying the health impact at national and local levels. Geneva: World Health Organization. 51 p.
13. Boschi-Pinto C, Velebit L, Shibuya K (2008). Estimating child mortality due to diarrhoea in developing countries. *Bull World Health Organ*, 86: 710–717.
14. Hutton G, Haller L, Bartram J (2007). Economic and health effects of increasing coverage of low cost household drinking-water supply and sanitation interventions to countries off-track to meet MDG target 10. Geneva: World Health Organization. 68 p.
15. Esrey SA. (1996). Water, Waste, and Well-Being: A Multicountry Study. *Am J Epidemiol;* 143:608-23
16. Checkley W, Gilman RH, Black RE, Epstein LD, Cabrera L, Sterling CR, Moulton LH (2004). Effect of water and sanitation on childhood health in a poor Peruvian peri-urban community. *Lancet;* 363: 112–18

17. Esrey SA, Habicht JP, Casella G (1992). The complementary effect of latrines and increased water usage on the growth of infants in rural Lesotho. *Am. J. Epidemiol.* 135, 659–666.
18. Merchant AT, Jones C, Kiure A, Kupka R, Fitzmaurice G, Herrera MG & Fawzi WW (2003). Water and sanitation associated with improved child growth. European Journal of Clinical Nutrition. 57, 1562–1568.
19. Lunn PG, Northrop-Clewes CA, Downes RM (1991). Intestinal permeability, mucosal injury and growth faltering in Gambian infants. *Lancet 338:* 907--910.
20. Campbell DI, Elia M, Lunn PG (2003). Growth faltering in rural Gambian infants is associated with impaired small intestinal barrier function, leading to endotoxemia and systemic inflammation. *J Nutr*; 133:1332–8.
21. Humphrey JH (2009). Child undernutrition, tropical enteropathy, toilets, and handwashing. *Lancet*, 374: 1032-1035.
22. Haghighi P, Wolf PL (1997). Tropical sprue and subclinical enteropathy: a vision for the nineties. *Crit Rev Clin Lab Sci*; 34: 313–41.
23. Gong YY, Cardwell K, Hounsa A, Turner PC, Hall AJ, Wild CP (2002). Dietary aflatoxin exposure and impaired growth in young children from Benin and Togo: cross sectional study. *BMJ*; 325:20 –1.
24. Gong YY, Egal S, Hounsa A *et al.* (2003). Determinants of aflatoxin exposure in young children from Benin and Togo, West Africa: the critical role of weaning. *Int J Epidemiol*; 32: 556–62.
25. Gong YY, Hounsa A, Egal S, Turner PC, Sutcliffe AE, Hall AJ, Cardwell K, Wild CP (2004). Post-weaning exposure to aflatoxin results in impaired child

growth: A longitudinal study in Benin, West Africa. *Environ Health Perspect;* 112:1334–8.

26. Khlangwiset P, Shephard GS, Wu F. Aflatoxins and growth impairment: a review (2011). *Critical Reviews in Toxicology*; 41(9): 740-755.
27. Smith LE, Stoltzfus RJ, Prendergast A (2012). Food chain mycotoxin exposure, gut health, and impaired growth: a conceptual framework. *Adv. Nutr***.** 3: 1–6.

CHAPTER 2

FORMATIVE RESEARCH ON HYGIENE BEHAVIOURS AND GEOPHAGY AS PART OF INTERVENTIONS TO IMPROVE INFANTS' GROWTH IN ZIMBABWE

ABSTRACT

Existing water, sanitation and hygiene interventions have not been designed to adequately protect the youngest children at greatest risk of morbidity and malnutrition. We conducted in-depth direct observation of 23 caregiver-infant pairs for a total of 130 hours and recorded hygiene behaviors to identify pathways of fecal-oral transmission of bacteria among infants. Hand washing with soap was not common for the mothers and infants. The most common vectors going into infants' mouths were infant's fingers, food, food service items, drinking water and toys. Infants' fingers and food were described as visibly dirty 75 % and 32 % of the times they went into the mouth, respectively. In addition, three infants actively ingested 11 ± 9 handfuls of soil (mean ±SD) and two ingested chicken feces 2 ± 1 times in 6 hours. *E. coli* contamination was found in mother's dominant hand in 11 (50 %) households, in drinking water from half of the households, and in kitchen floors and yard soil samples from more than half of the households. We estimate that an infant ingesting 1 g chicken feces and 20 g of soil from a laundry area of the kitchen yard would consume 4.7-23 M and 440-4,240 viable cells of *E. coli*, respectively, from these sources. In addition to the standard WASH and nutrition interventions, infants in low-income communities should be protected from

exploratory ingestion of chicken feces, soil and geophagia for optimal child health and growth.

INTRODUCTION

Poor growth during infancy and early childhood remains an important risk factor for childhood morbidity and mortality and a major public health challenge in low and middle income countries. Childhood stunting is a risk factor for diminished survival, short adult height, impaired intellectual development, reduced economic productivity and low offspring birth weight (1). Globally, approximately 178 million children under 5 years of age are stunted, with an estimated 35% of child deaths attributed to sub-optimal nutrition (2). In Africa and Asia height-for-age Z scores decline from near zero at birth to –2.0 by 18 months of life. Rigorous dietary interventions have improved stunting by 0.7 Z scores at best, only one-third of the average deficit (3).

Environmental Enteropathy (EE), a chronic subclinical intestinal pathology, is common among infants in low income countries and has been proposed as a major pathway to childhood stunting (4). Although diarrhea, the second leading cause of child mortality word wide (5), causes malnutrition, prevalent diarrhea is not always associated with poor growth in the long term because of catch-up growth between episodes (6). EE, which is characterized by reduced intestinal barrier function and chronic systemic inflammation, may be a more important cause of poor growth in children than diarrhea (7). Research in the Gambia showed that 43% of linear growth failure could be explained by increased intestinal permeability (IP), a measure of EE; in contrast, the prevalence of diarrhea was not associated with growth failure (8). Although the precise

cause of EE is unknown, it has been linked to unsanitary and unhygienic living conditions, and is likely to arise from chronic, subclinical exposure to fecal pathogens.

The primary pathways of fecal-oral transmission have been described for decades using the "F Diagram", food, flies, fingers, field and fluids, (9), yet the relative importance of each path way is not known. Furthermore, the primary routes of transmission may be different for infants and young children, because their primary food and fluid is breast milk and who regularly mouth objects as part of normal development. In developing countries, young children crawl and play in areas where they may come into contact with soil that is contaminated with human and animal feces. Human or animal feet that tread in feces deposited in the open bring pathogens into the domestic environment-home and immediate vicinity to infants and young children (10). In Lima, Peru, an in-depth behavioral observation study of 21 children under five years of age reported a mean of 3.9 (SD 4.6) episodes of ingestion of chicken feces during a 12 hour period (11). A recent comprehensive review reported that human geophagy (intentional consumption of earth) is common among children and pregnant women in low income countries, where pathogen densities are highest (12). Therefore, exposure to fecal bacteria among children in low income countries might be greater than has been reported in studies that focused only on food and drinking water contamination.

During formative research to inform the design of interventions to improve infant growth, we carried out a study to identify the major pathways of fecal-oral microbial transmission among infants in rural Zimbabwe. The study was executed in two phases: I. Observation of infants and their caregivers to identify the frequency of all objects put into infants' mouths (whether swallowed or not) and presence of visible dirt

over a 6-hour period per household; II. Sampling and microbiological analysis of the objects that were mouthed most frequently and/or were most visibly dirty. To ensure uniform understanding among the research staff during data collection, prior training was done and the same team was involved in the pre-testing of the tools and developing detailed field guides.

METHODS

Study Site and Sample Frame

The study was conducted in rural Shurugwi District, Midlands Province, Zimbabwe. Most households in this district subsist through small-scale farming, poultry and cattle keeping. Twenty one households with 7 infants in each age group (3-6 months, 6-12 months, and 12-18 months) were selected from Village Health Workers' lists. An additional two households with infants less than 3 months old were also included. The households consisted of two or more traditional mud or brick walled houses. Majority of the houses had roofs made of thatch and half had earthen or cow dung smeared floors. The compounds were open yards with bare loose sandy soil and no fence. Infants were free to crawl on bare soil where poultry and other animals were free to roam.

Ethics

On the first visit to each household, the study was introduced by the field supervisor and written informed consent to participate in the study was obtained from mothers. Ethical approval to conduct this study was granted by the Medical Research Council of Zimbabwe and the Institutional Review Boards of Johns Hopkins University

Bloomberg School of Public Health and the Research Institute of McGill University Health Centers.

Caregiver-Infant Observation

During the observation phase in February 2011, two trained researchers conducted a 6 hour in-depth observation between 8.30 AM and 2.30 PM in each of the 21 households with 6-18 months old. (Observation was stopped at 2 hours in the two household with less than 3 months old since these infants were mostly protected and inactive). One researcher used a pre-tested semi-structured data collection tool to record every object that was either touched or mouthed by an infant, whether the object was visibly dirty or not, and the frequency of object-mouth episodes. Researchers also observed and recorded the mother's hand washing behavior and any infant nappy change and hand washing. The potential triggering events considered for hand washing were defined as after toilet use, after contact with animal stool, before feeding the baby, before handling food, before eating and after sweeping the floor or yard.

After briefly interviewing the mother after arrival at the household, the second researcher conducted 2 hour interval spot checks and recorded the number of corralled and roaming animals and the presence of fecal material in the kitchen yard using a pre-tested structured questionnaire. The spot checks also involved inspecting and recording the cleanliness of the mother's and infant's hands. The second researcher also determined whether the household had a hand washing station and functional latrine, and whether there was evidence of their recent use. To maintain the quality of data, debriefing sessions were held daily after every household visit. Researchers re-checked

and cross-checked questionnaires and recording of key events and behaviors to maintain consistency in data collection.

Following all infant observations, data were analyzed to identify the key potential vectors, defined as i) the objects mouthed most frequently; ii) the objects that were ever mouthed and were most visibly dirty. The objects identified during Phase I by the frequency definition were infant, mother, and sibling hands; food; water; toys/play objects (e.g. plastic cans and dolls); and food serving utensils; the objects identified by the visibly dirty definition were soil and chicken feces.

Microbiological analyses (May--June 2011)

Two subsequent visits were made at 10 day intervals to the households visited in Phase 1 to collect samples or swabs of the key potential vectors identified. (A total of 22 households were sampled because one mother was away during the sampling phase). Three research staff with experience in aseptic sampling techniques and microbiological analysis collected fourteen different samples from each household during mid-morning hours.

Soil samples were collected from three parts of the yard where children were most likely to play and likely point sources of fecal contaminated soil where crawling children have access: areas used for laundry, nappy changing and bathing; the kitchen door step, and the edge of the rubbish pit or path to the rubbish pit (if the pit was distant). On the second sampling visit, an additional soil sample was also collected from the field cultivated by the mother to determine if infants were exposed to fecal bacteria. Except for the initial sampling visit to the first four households, mothers and village

health workers were not aware of the day and time of visits for sample collection, to minimize social desirability bias.

Environmental samples (infant cup, infant toys and spoon and kitchen floor area) and hand swabs (mother's and sibling's dominant hand) were taken using commercial environmental sponge sampling kits, Bacti Sponge Kit, Hardy Diagnostics, Santa Maria, CA (13). Four swabs were also taken from the child's left palm, right palm, right fingers and left fingers at each visit. About 20 g of food, water, soil and chicken feces samples were collected into sterile screw cap bottles using an aseptic technique. The mother was requested to scoop food during infant feeding into a sterile screw cap bottle. If no infant feeding occurred during the sampling visit, the mother was requested to scoop any leftover food that was fed to the child using the same spoon used for feeding. The procedure at the second visit replicated the first visit. A fixed area was sampled from the kitchen floor (equivalent to four sponge kits sizes; 128 cm^2), and from the infant cup and spoon (equivalent to 6 sponge sizes; 192 cm^2). Infant toys were of different sizes and shapes and therefore; swabs were taken from a fixed area of surfaces that could easily fit into an infant's mouth (equivalent to 2 sponge kit sizes; 64 cm^2).

After sampling, swabs were immediately placed in cooler boxes, covered with ice packs and transported to the field laboratory for microbiological analysis within 12 hours. All samples were analyzed for total aerobic counts (AC), *E. coli*/coliforms (EC) and *Enterobacteriaceae* (EB) using 3M^{TM}Petrifilm^{TM} prepared plates (3M Microbiology, St. Paul, MN) according to the manufacturer's instructions (14). Ninety nine ml of sterile Butterfield's phosphate buffer was added to 11 g of food, soil or chicken feces samples in sterile bags with filters and homogenized using a hand roller

for 3 min to prepare a 1:10 dilution. Subsequent dilutions were prepared from the filtrate. Food and water samples were diluted (1:10--1:100,000) with sterile Butterfield's phosphate buffer. 1mL of each sample dilution was plated in duplicate onto each of the 3 Petrifilm products and incubated at $35^{o}C \pm 1^{o}C$ for either 24 ± 2 h (for coliforms and *Enterobacteriaceae*) or 48 ± 3 h (for *E. coli* and total aerobic counts).

Sponges from the hand and environmental swabs were transferred aseptically to homogenizer bags, to which 20 mL of Butterfields phosphate buffer was added and squeezed for 1 minute using a hand roller homogenizer. 1 ml of the resulting solution was plated in duplicate directly onto the EB and EC Petrifilms and incubated as described above. Further 1:10 dilutions for the hand swabs and environmental surfaces swabs were undertaken for the AC enumeration.

Data Analysis

The frequencies of all objects put in the infant's mouths were calculated and the 10 most frequently mouthed and the dirtiest objects were taken as the potential key vectors for phase II bacteriologic analyses. Geometric means for bacterial populations in each potential vector were calculated as colony-forming units per gram (CFU/g). All summary statistics and t- test comparisons were carried out using STATA statistical software, Version 10 (College Station, Texas, USA).

RESULTS

Household Characteristics

The researchers observed 23 caregiver-infant pairs were observed for a total of 130 hours during the infant observation phase. Baseline characteristics of households

are shown in **Table 2.1**. All except one mother were married and had attained at least some level of primary education. One-third of the households did not have a latrine. Hand washing stations and soap were found in about half of the households. More than half of the households (56%) had a borehole as their primary water source. Other households used either a protected or unprotected wells.

General Hygiene

At the beginning of the infant observation phase one third of the mothers had visibly dirty hands. Seventeen percent of the infants had visibly dirty hands (**Table 2.2**). The area where the child played was not recently swept in 16/23 (70%) households. The kitchen floor was made of cow dung or dirt in half of the households and animals (mostly poultry) were observed in the kitchens of a third of households on arrival. One third of households had chicken feces on the kitchen floor and the majority (87%) had chicken feces in the kitchen yard. Other animal feces were also observed in 7 (30%) households. No human feces were observed in any yard. Chickens (median 10; range 1-31) were found freely roaming in the kitchen yard of 15--21 households. Similarly, guinea fowl (median 8; range 2-18) were freely roaming in the yard in 4-7 households.

Hand washing and nappy change practices

Overall mothers washed hands 109 times out of 250 triggering events (44%) but used soap only 7 times (6%). Mothers washed their hands after toilet use, contact with animal stool, or nappy changing on17/53 (32%) occasions, but used soap in only 4 of these 17 hand washing (24%) . The mothers also washed their hands before 19 of the 44

(43%) breastfeeding episodes, but did not use soap on any of these hand washing. Air-drying of hands was common but use of running water during hand washing was not (**Table 2.3**).

During 130 hours of observation, infant hands were washed 21 times: 8 of these were as part of a bath, 6 of which included soap (**Table 2.4**). Soap was not used in any of the 13/21 (62%) cases of infant hand washing which were not part of a bath; most of these (8/13) occurred just before feeding (Table 2.4). Hand washing was more common for12-18 month old and in this age group none of the hand washing events was during a bath. Among 3-12 months-old infants, hand washing occurred during a birth.

Mothers washed their hands after 13 of the 41 (32%) nappy changes observed and used soap in only 4 occasions. Of the 25 nappy changes involving feces, nappy water was either emptied into a latrine (4 times), yard (5 times) or a rubbish pit (12 times). Two of the 25 times feces were buried in the garden and in two other cases; the observer was not able to see the method of disposal. Most commonly mothers did nappy washing at the same spot/area (usually in the shade) where they did laundry.

Frequency of infant mouth contact of potential fecal-oral vectors

Objects identified as major feco-oral vectors by the frequency definition were infant, mother, and sibling hands; food; water; toys/play objects; and food service utensils. The objects identified as major feco-oral vectors by most visibly dirty definition were soil and chicken feces (**Table 2.5**). Infant hands were put into the mouth; mean (SD) 38 (38.9) times between the 20 infants, and were visibly dirty on the majority (75%) of these episodes. Mothers' hand was put into infants' mouth less

frequently; mean (SD) 11.3 (11.1) times, in only 3 households, but were always visibly dirty (Table 2.5). Three infants ingested soil, mean (SD); 11.3 (9.2) times and 2 infants ingested chicken feces, mean (SD); 2 (1.4) times (Table 2.5). Two of the three infants who mouthed soil also took stones into their mouth.

Table 2.1: Maternal and household characteristics of 23 mother-infant dyads in rural Zimbabwe

Maternal Characteristics	n(percent) or mean ±SD
Caretaker	
Mother	22(95.7)
Grandmother	1(4.3)
Mother's age (years)	25.66 ± 6.5
Marital status	
Married	22(96)
Single	1(4)
Education Level	
Primary education	5(22)
Secondary education	18(78)
Number of Children	
Under 18	3.74 ± 2.0
Under 5	1.56 ± 0.8
Household Characteristics	
Latrine Ownership	
Own	10(43)
Neighbor's	5(22)
Do not use/ do not have	8(35)
Hand washing	
Hand washing station	13(56)
Soap at hand washing station	10(44)
Wet ground at hand washing station	10(44)
Primary water sources	
Borehole	13(57)

Table 2.1: Maternal and household characteristics of 23 mother-infant dyads in rural Zimbabwe

Maternal Characteristics	n(percent) or mean ±SD
Protected well	8(35)
Unprotected well	1(4)
River	1(4)
Scooping container at point of use	
Specific scooping cup	9(39)
Any cup	13(56)

Table 2.2: General hygiene characteristics of infant's environment during infant observation

Characteristics	On arrival*
Caregiver's hands visibly dirty	7 (30)
Baby's hands visibly dirty	4 (17)
Diaper's or child's bottom not clean	1 (4)
Stagnant water within infant's reach**	7 (30)
State of the kitchen	
There are unwashed utensils	9 (39)
There are uncovered utensils	0 (0)
There is uncovered food	1 (4)
Smooth concrete kitchen floor	12 (52)
Dirt or cow dung kitchen floor	11 (48)
Spill on the kitchen floor, food or drink	5 (23)
Poultry feces visible on the kitchen floor	8 (35)
There are animals in the kitchen	8 (35)
State of the outside yard	
Kitchen yard swept	16 (70)
Area where child plays is swept	16 (70)
There are poultry feces visible on yard	20 (87)
There are human feces visible on the yard	0 (0)
There are animals feces visible on the yard	7 (30)

*Number (%).

**Any stagnant water within infant's reaches in the kitchen or outside yard

Table 2.3: Mother's hand washing practices, number of events (%)

Key events	Opportunities	Any hand washing*	HWWS**	Running water	Air drying†
Possible contact with feces					
After Adult Toilet	10	4 (40.0)	0 (0)	1 (25)	4 (100)
Contact with animal stool	2	0 (0)	0 (0)	0 (0)	0 (0)
After nappy change[#]	41	13 (32)	4 (10)	2 (15)	13 (100)
Sub total	53	17 (32)	4 (8)	3 (18)	17 (100)
After sweeping floor or yard	36	11 (31)	0 (0)	1 (9)	10 (91)
Before feeding the baby	32	14 (44)	0 (0)	1 (7)	10 (71)
Before handling food	51	23 (45)	1 (2)	3 (13)	22 (96)
Before eating	23	14 (61)	0 (0)	3 (21)	14 (100)
Before breastfeeding	44	19 (43)	0 (0)	0 (0)	16 (84)
Others‡	11	11 (100)	2 (2)	0 (0)	11 (100)
Total	250	109 (44)	7 (3)	11 (10)	100 (92)

*Hand washing with sand was done once. **HWWS- hand washing with soap. The denominator for HWWS and subsequent columns is the number of times of any hand washing.

†Dried using a visibly dirty towel 4 times and a not visibly dirty towel 5 times.

Table 2.3 (Continued)

#25 nappy changes were due to defecation and only 4 times were feces or nappy wash waste water disposed of in latrine. The four events of latrine disposal of feces were one for each of the age categories < 3 and 3-6, and two for 12-18 months. ‡Others include: before milking the cow, after milking, after tethering goats, after feeding the child, after eating, after putting the baby to sleep and after changing clothes.

Table 2.4: Infants' hand washing practices

Age category (months)	< 3*	3-6	6-12	12- 18	All
N	2	7	7	7	23
Number of hand washing**	0.5 (0--1)	0(0--1)	1 (0--1)	1 (0--4)	1(0--4)
Total hand washing	1	3	4	13	21
As part of bath	1	3	4	0	8
HWWS	0	2	4	0	6
Number wiped or washed**	0.5 (0-1)	1 (1-1)	1 (0-2)	1 (0-5)	1 (0-5)
Total wiped or washed	1	7	7	15	30

*Age category < 3 months had two infants observed for 2 hours each and only one hand washing event during bath. **Number of hand washing, wiped or washed is median (range).

Table 2.5: Key potential vectors for feco-oral transmission of bacteria

Potential vector	n (% of total)	Vector-mouth episodes (mean ±SD)	% Visibly Dirty*
Food**	19 (83)	90.7 ± 70.5	32 (6/19)
Baby's hands	20 (87)	38.0 ± 38.9	75 (15/20)
Baby's cup and spoon	20 (87)	33.6 ± 20.2	25 (5/20)
Fresh fruits†	12 (52)	13.3 ± 10.3	58 (7/12)
Toys	13 (57)	13.3 ± 8.0	54 (7/13)
Mother's hands	3 (13)	11.3 ± 11.1	100 (3/3)
Soil	3 (13)	11.3 ± 9.2	100 (3/3)
Breasts	18 (78)	7.9 ± 4.5	0 (0/18)
Sibling's hands	3 (13)	7.3 ± 5.5	100 (3/3)
Water	10 (44)	3.2 ± 2.0	30 (3/10)
Stone	4 (17)	3.0 ± 2.7	100 (4/4)
Chicken feces	2 (9)	2.0 ± 1.4	100 (2/2)

* In brackets are number of episodes/ number of infants or households where episodes occurred.

Table 2.5 (Continued)

**Food: referred to home cooked food: Porridge, *Sadza*, bread, milk, green maize, beans, fat cook, sweet potato, pumpkin, eggs, nuts, crisps, beef, fish and green peas.

†Fresh fruits: Guava, mangoes and sweet reeds, though the latter is not a fruit *per se*.

Bacterial contamination of the key vectors

All chicken feces samples were positive for *E coli*. Compared to other potential vectors, chicken feces had far higher counts of both *E coli* and total coliforms (**Tables 2.6 and 2.7**). All soil samples from the kitchen yard and within reach of a crawling infant were also commonly and highly contaminated with *E. coli* (Table 2.6). Water samples were positive for *E.coli* in more than half of the households. However, on a per gram basis, *E. coli* counts were 3-35 folds higher in soil compared to water. Total coliforms, *Enterobacteriaceae*, and total aerobic counts were respectively 15-104, 24-110 and 88-947 folds higher in soil compared to water, based on 95% confidence intervals of the geometric means of bacterial counts. None of the food samples tested positive for *E. coli* and counts of the other bacteria were lower in food than in all other potential vectors. Mother's and infant's hand were contaminated with *E. coli* in 50% and 13% of households, respectively. The infant's cup and spoon cultures yielded *E. coli* in 5 (23%) households. Kitchen floor swabs were positive for *E. coli* in 18 (82 %) households. Mean *E coli* count/swab from kitchen floors did not differ for those made of cow dung (6,030 ± 22,286) compared to cement (5,705 ± 21,371), (p =0.96).

Table 2.6: Overall mean and number of samples (%) in each category of *E. coli* counts

		E. coli positive, n (%)		*E. coli*	Number of samples under each category of counts n (%)			
Vector	Samples (n)	Samples positive	Households positive	Mean (95 % CI) *	< 100	100--10,000	10,000--1000,000	>1000,000
Food (porridge)	15	0 (0)	0 (0)	0(0-0)	0 (0)	0(0.0)	0(0)	0(0)
Water	43	14 (33)	12 (55)	2(1-3)	13 (30)	1 (2)	0 (0)	0 (0)
Breast	36	0 (0)	0 (0)	0(0-0)	0 (0)	0(0.0)	0(0)	0(0)
Hand swabs								
Index child's left fingers	37	4 (11)	3 (14)	1(0-2)	4 (11)	0(0.0)	0(0)	0(0)
Index child's left palm	36	2 (6)	2 (9)	1(0-2)	2 (6)	0(0.0)	0(0)	0(0)
Index child's right fingers	37	2 (5)	2 (9)	1(0-2)	1 (3)	1 (3)	0(0)	0(0)
Index child's right palm	37	1 (3)	1 (5)	1 (0-2)	1 (3)	0(0)	0(0)	0(0)
Siblings dominant hand	20	1 (5)	1 (5)	1 (0-2)	1 (5)	0(0)	0(0)	0(0)
Caregiver's dominant hand	43	13 (30)	11 (50)	4 (2- 8)	9 (21)	3 (7)	1 (2)	0(0)
Environmental Samples								
Index child's cup and spoon	40	7 (18)	5 (23)	2 (1- 4)	4 (10)	3 (8)	0 (0)	0(0)
Kitchen floor	42	25 (60)	18 (82)	42 (14- 130)	6 (14)	14 (33)	5 (12)	0(0)
Soil								
Field soil	22	1 (5)	1 (5)	1 (0- 2)	1 (5)	0(0)	0(0)	0(0)
Trodden path to pit	43	17 (40)	14 (64)	5 (3 - 8)	12 (28)	5 (12)	0(0)	0(0)
Kitchen door step	43	24 (56)	16 (73)	17(7- 43)	9 (21)	15 (34)	0(0)	0(0)
Laundry area	43	30 (70)	18 (82)	69 (22- 212)	10 (23)	16 (37)	4 (9)	0(0)
Chicken feces	42	22 (100)	22 (100)	1.03e+07 (4.7e+06- 2.26e+07)	0 (0)	1 (2)	7 (17)	34 (81)

*Mean counts are geometric means (95 % confidence interval) cfu/g or cfu/swab for Breast, hand swabs and environmental samples. Number of households, n = 22.

Table 2.7: Overall mean and number of samples (%) in each category of other bacteria counts

Coliforms			Number of samples under each category of counts n (%)			
Vector	Samples (n)	Mean (95% CI) *	< 100	100--10,000	10,000--1000,000	>1000,000
Food (porridge)	15	2(0--6)	14 (93)	1 (7)	0(0)	0(0)
Water	43	18(10--33)	36 (84)	7 (16)	0(0)	0(0)
Breast	36	1(0--2)	35 (97)	1 (3)	0(0)	0(0)
Hand swabs						
Index child's left fingers	37	7 (3--18)	29 (78)	8 (22)	0(0)	0(0)
Index child's left palm	36	3 (1--6)	33 (92)	3 (8)	0(0)	0(0)
Index child's right fingers	37	11(5--25)	29 (78)	8 (22)	0(0)	0(0)
Index child's right palm	37	3 (2--6)	35 (95)	1 (3)	1 (3)	0(0)
Siblings dominant hand	20	11(3--47)	14 (70)	5 (25)	1 (5)	0(0)
Caregiver's dominant hand	43	71 (30--166)	22 (51)	19 (44)	2 (5)	0(0)
Environmental Samples						
Index child's cup and spoon	40	23 (9--62)	25 (63)	14 (35)	1 (3)	0(0)
Kitchen floor	42	658 (324--1340)	9 (21)	29 (69)	4 (10)	0(0)
Soil						
Field soil	22	7 (2--24)	16 (73)	6 (27)	0(0)	0(0)
Trodden path to pit	43	274 (126--596)	12 (28)	28 (65)	3 (7)	0(0)
Kitchen door step	43	639 (294--1,390)	9 (21)	29 (70)	6 (4)	0(0)
Laundry area	43	1,880(718--4,950)	5 (12)	22 (51)	16 (37)	0(0)
Chicken feces	42	2.32e+07(1.01e+07--5.34e+07)	0(0)	0(0)	5 (12)	37 (88)
Enterobacteriaceae						
Vector	Samples (n)	Mean (95% CI) *	< 100	100--10,000	10,000--1000,000	>1000,000
Food (porridge)	15	4(1--13)	14 (93)	1 (7)	0(0)	0(0)
Water	43	52(27--100)	29 (67)	13 (30)	1 (2)	0(0)
Breast	36	3(2--6)	33 (92)	3 (8)	0(0)	0(0)
Hand swabs						
Index child's left fingers	37	47(18--126)	18 (49)	19 (51)	0(0)	0(0)
Index child's left palm	36	14 (6--37)	26 (72)	10 (28)	0(0)	0(0)
Index child's right fingers	37	37 (14--95)	21 (57)	15 (41)	1 (3)	0(0)
Index child's right palm	37	12 (5--27)	28 (76)	8 (22)	1 (3)	0(0)
Siblings dominant hand	20	92 (21--400)	9 (45)	10 (50)	1 (5)	0(0)

Table 2.7: Overall mean and number of samples (%) in each category of other bacteria counts

Enterobacteriaceae						
Vector	Samples (n)	Mean (95% CI) *	< 100	100--10,000	10,000--1000,000	>1000,000
Caregiver's dominant hand	43	299 (137--656)	9 (21)	33 (77)	1 (2)	0(0)
Environmental samples						
Index child's cup and spoon	40	96 (33--284)	17 (43)	21 (53)	2 (5)	0 (0)
Kitchen floor	42	1,850 (1,030--3340)	2 (5)	35 (83)	5 (12)	0 (0)
Soil						
Field soil	22	40 (11--149)	13 (59)	8 (36)	1 (5)	0 (0)
Trodden path to pit	43	1,290 (623--2, 690)	3 (7)	34 (79)	6 (14)	0 (0)
Kitchen door step	43	2,970 (1,390--6,350)	3 (7)	28 (64)	13 (30)	0 (0)
Laundry area	43	5,750 (2,560--12,900)	2 (5)	24 (56)	16 (37)	1 (2)
Chicken feces	42	2.95e+07 (1.43e+07--6.07e+07)	0(0)	0(0)	3 (7)	39 (93)
Aerobic counts						
Vector	Samples (n)	Mean (95% CI)*	< 100	100--10,000	10,000--1000,000	>1000,000
Food (porridge)	15	1,420(168--12,000)	3 (20)	8 (53)	2 (13)	2(13)
Water	43	21,400(10,200--45,100)	0(0)	19 (44)	19 (44)	5 (12)
Breast	36	54,800(40,800--73,600)	3 (8)	32 (89)	1 (3)	0 (0)
Hand swabs						
Index child's left fingers	37	86,100 (72,000--104,000)	0(0)	0(0)	36 (97)	1 (3)
Index child's left palm	36	66,300(51,400--85, 600)	0(0)	1 (3)	35 (97)	0 (0)
Index child's right fingers	37	72,600 (58,600--90,100)	0(0)	0(0)	37 (100)	0 (0)
Index child's right palm	37	65,000 (51,100--82,700)	0(0)	1 (3)	36 (97)	0 (0)
Siblings dominant hand	20	90,300 (71,000--115,000)	0(0)	0(0)	20 (100)	0 (0)
Caregiver's dominant hand	43	77,700 (55,400--109,000)	0(0)	2 (5)	41 (95)	0 (0)
Environmental Samples						
Index child's cup and spoon	40	50,000(36,000--69,800)	0 (0)	2 (5)	38 (95)	0 (0)
Kitchen floor	42	105,000 (94,600--117,000)	0(0)	0(0)	42 (100)	0 (0)
Soil						
Field soil	22	1.02e+06 (573,000--1.82e+06)	0 (0)	0 (0)	9 (41)	13 (59)
Trodden path to pit	43	1.89e+06 (1.23e+06--2.92e+06)	0 (0)	0 (0)	13 (30)	30 (70)
Kitchen door step	43	4.44e+06 (2.49e+06--7.93e+06)	0 (0)	0 (0)	8 (18)	35 (81)
Laundry area	43	2.03e+07 (1.16e+07--3.55e+07)	0 (0)	0 (0)	2 (5)	41 (95)

Table 2.7: Overall mean and number of samples (%) in each category of other bacteria counts

Aerobic counts						
Vector	Samples (n)	Mean (95% CI)*	< 100	100--10,000	10,000--1000,000	>1000,000
Chicken feces	42	1.30e+09 (4.74e+08--3.58e+09)	0 (0)	0 (0)	1 (2)	41 (98)

*Mean counts are geometric means (95 % confidence interval) cfu/g or cfu/swab for Breast, hand swabs and environmental samples. Number of households, n = 22.

Table 2.8: Infant feces (nappy wash waste water) disposal

	Age group				
Fecal disposal	<3	3--6	6--12	12--18	Total n (%)
Garbage/pit	1	4	4	3	12(48)
Tossed in Yard	0	1	3	1	5(20)
Latrine	1	1	0	2	4(16)
Buried in garden	0	0	0	2	2(8)
Not seen	1	1	0	0	2(8)
Total, n (%)	3(12)	7(28)	7(28)	8(32)	25(100)

DISCUSSION

In this study, we show that infants in rural Zimbabwe are frequently exposed to fecal indicator bacteria through daily activities. Caregiver and infant hands, contaminated water, and ingestion of soil and chicken feces were the main vectors of feco-oral transmission during infancy. In this study, fecal contamination is represented by the frequency of *E. coli,* the classical inhabitant of the intestinal tract of warm-blooded animals. We hypothesize that frequent ingestion of fecal bacteria may cause environmental enteropathy, which is a major hypothesized cause of poor growth in early life.

Optimal hand washing practices were uncommon among the caregivers and infants observed. Mothers hand washing of infants' hands was rare in this rural setting were constantly exposed to fecal bacteria from crawling on cow dung smeared kitchen floors and on bare soil in the yard with heavy exposure to poultry feces. Hand washing for older infants was only done during bathing. Even though most caregivers had some level of secondary education, hand washing with soap (HWWS) was rarely observed

after animal or human fecal contact. The observed rate of hand washing with soap after fecal contact (7%) was much lower than that reported in a study from Bangladesh based on 5 hours of structured observation for HWWS occurred after adult caregiver defecation, 33% (15) and 23% after adult caregiver's defecation and cleaning a child's bottom (16) and less than half the rate reported (17%) in a comprehensive review of formative research studies carried out in 11 low income countries (17). Similar low rate of HWWS after fecal contact is reported in several other low income countries (18).

The presence of either water or soap at a hand washing station doubled HWWS after fecal contact in Bangladesh (16). However, even though a hand washing location was identified in 57% of the households and soap in 43% of the households in this study, HWWS after fecal contact was strikingly low in our study. The low frequency of HWWS could be related to unavailability of water at a hand washing location in the study households (0%), which was an important trigger in Bangladesh. However, hand washing behavior is driven by several factors. A review of 11 studies done in Africa, Asia and Latin America identified three kinds of hygiene behaviors: habitual, motivated and planned (17). Hygiene habits were learnt at an early age but soap use was rarely taught by parents and school. Key motivations for hand washing were disgust from contaminated hands and social norms. Other motivations included comfort and nurture (the desire to care for one's children). Planned hand washing with an aim to prevent disease transmission was rare. Plans involving hand washing included; to improve family health and to teach children good manners. In this review (17), soap and water was available in almost every household and yet only 17% of the caretakers washed

hands with soap after the toilet. This underscores that environmental barriers may not account entirely for the low rate of HWWS in our study.

Although food did not appear to be a critical source of fecal indicator bacteria in this study, the infant feeding environment was frequently contaminated with fecal matter. The kitchen floor, where infant feeding usually takes place, was frequently contaminated with *E. coli* and in part chicken feces. Although hand washing with soap can be effective in breaking the fecal-oral pathway, it was uncommon. Most mothers did not dry their infant's hands or restrain the baby after hand washing. It was common for infants to place their wet fingers on the kitchen floor or on bare soil, and then put their soiled fingers into their mouths soon after. In addition, food, fruits and objects were often picked straight up from the floor into an infant's mouth. Infant hand washing might increase the risk of fecal bacteria exposure if thorough drying of the hands and subsequent protection from dirt is not adhered to.

A substantial minority of infants actively ingested soil and chicken feces or licked stones from the bare yard soil. Most notably caregivers did not stop the infants from active soil ingestion. Two subsequent FGDs in rural Zimbabwe confirmed that babies eat soil and either fresh or dried chicken feces (Zvitambo qualitative research, unpublished). Some mothers reported that in-laws or village elders advised soil eating, because it was good for the baby's intestines or treated stomach illnesses.

Ingestion of chicken feces (overall mean, 0.2 episodes per child) was less frequent than that reported among Peruvian toddlers, 3.9 episodes per child (11). Nevertheless, from our structured observation, a one year old infant in rural Zimbabwe may typically ingest up to 1 gram chicken feces in a day, 20 grams of laundry area soil

and 400 ml of water from a contaminated source and as a result consumes 4,700,000--23,000,000,440--4,240 and 400--1,200 *E. coli* counts, from these sources respectively, based on the 95% confidence intervals (Table 2.6). In the Peruvian study, viable *Campylobacter jejuni*, an important cause of dysenteric diarrhea, was isolated from infected chicken's feces up to 48 hours after deposition (11), and in our own study all chicken feces contained *E. coli* regardless of the time of deposition. Ingestion of chicken feces and soil containing chicken feces therefore represents a huge potential burden of pathogenic bacteria, which may cause diarrhea in addition to propagating EE.

Water contamination and handling of food post-cooking by the mothers also puts infants at risk of fecal bacteria ingestion. In an earlier study in Zanzibar, Tanzania, bacterial counts in traditional complementary foods did not vary by food groups suggesting that the level of contamination was typical of a particular household and not unique to food type (14). Contamination of food is largely dependent on food hygiene practices and storage time (19, 20). However, there is little convincing epidemiologic evidence of the importance of food hygiene in in diarrheal pathogens transmission in developing countries. This may be due to the importance of other sources like unsafe feces disposal (10). Therefore, optimal food handling and storage practices might not effectively prevent fecal bacteria exposure if the domestic environment is frequently contaminated. Fecal Indicator bacteria (FIB) contamination of mothers' and children's hands was associated with FIB contamination in stored drinking water in Dar es Salaam, Tanzania (21) which was more contaminated than source water suggesting that water was re-contaminated by hands at the point of use. Such an association could

explain the contamination of drinking water with *E. coli* in half of the households in this study.

Half of the caregivers' dominant hands were positive for *E. coli* in a context where hand washing with soap after fecal contact was rarely practiced. Similar fecal contamination frequency of mothers and children hands was also reported in Tanzania (21). These findings further strengthen the need to effectively break the fecal-oral transmission route via hands through effective interventions like HWWS (10).

Our study had several limitations. We only took one hand swab per caregiver or sibling per visit. The four swabs taken from parts of index child's hands were also taken once in one visit and were therefore not representative of a day's microbial exposure for the infant. A more extensive sampling strategy in future studies would enable variability in counts of fecal bacteria to be evaluated. In Tanzania, mother's hands were quickly re-contaminated with fecal bacteria, during household activities such as sweeping, following hand washing (22).

Samples were taken during the dry hot season. Fresh fruits which were common during infant observation could not be sampled. The dry hot winter days and the nature of the soil (sandy with less than 20% silt) was a less conducive environment for survival of fecal bacteria. The bacterial counts reported in this study could therefore be much lower than would be typical during the wet season. Many of the poultry feces samples were dry and could have been exposed to the sun for hours and therefore the counts reported for chicken feces could be lower than from fresh feces. These limitations, however, do not negate the implications of this study.

CONCLUSION

The study suggests that existing WASH interventions are failing to protect infants from ingesting soil and feces. Whereas WASH interventions to date have focused on hand washing, improved drinking water sources, point-of-use water treatment and improved sanitation, little attention has been given to exploratory ingestion of soil and chicken feces and geophagy. To our knowledge, no studies to date have quantified the burden of fecal bacterial ingestion by young children through geophagia, exploratory behavior or crawling on bare soil. These exposures place infants at risk of diarrheal diseases (from *Camplybacter jejuni*, enteropathogenic strains of *E. coli,* and *Salmonella* species, among others) and development of EE.

Effective interventions should be carefully designed to break this prominent route of fecal-oral transmission. Safe human feces disposal, a primary barrier to bacteria transmission, is critical in preventing pathogenic bacteria from reaching children's immediate environment (10). More attention should be devoted to interventions aimed at reducing animal fecal contamination of child's environment. This study points to a widespread cultural belief that soil ingestion is beneficial. Although ingestion of clay-like earth could be associated with reducing the effects of toxins or alleviating gastrointestinal distress following geophagia (12), the type of soil in rural Shurugwi district is coarse and sandy and unlikely to have any effective enterosorptive capacity.

This study demonstrates that existing evidence based WASH interventions will not effectively eliminate fecal-oral transmission of bacteria among infants and young children. New interventions and programs are needed to address these environmental health risks that potentially diminish the benefits achievable for child hood health and

growth from improved dietary interventions. A clear separation of the infant from the frequently contaminated soil without negating the child's physical and cognitive development, through restricting exploratory behavior, can reduce the risk from these environmental sources. Educating mothers on personal and environmental hygiene, safe disposal of human and animal feces should complement efforts to provide a clean environment for young children.

Further research is recommended to analyze for presence of fecal pathogens such as gastrointestinal viruses, diarrheagenic *E. coli,* and human specific *Bacteriodales* using highly specific molecular techniques. Microbial source tracking to identify the source of fecal contamination is necessary in these settings to provide evidence of the relative importance of animal and human feces in contaminating domestic environment. Studying the distribution of fecal bacteria and pathogens within the household environment can guide future interventions on improving domestic hygiene.

Acknowledgements

The authors thankfully acknowledge Marcia McKenzie of The Research Institute of the McGill University Health Centre and Nancy Carey of Department of Food Science, Cornell University for diligently assisting in purchasing and shipping of the microbiology laboratory reagents and supplies. We thank Rukundo Kambarami of the Division of Nutritional Sciences, Cornell University and Dadirai Fundira of Zvitambo Research Project, for their dedicated efforts in Infant Observation and data collection. We also thank the study participants. The objectives of this research could

not have been achieved without the laboratory space and facilities at Shurugwi District Hospital availed by the Ministry of Health and Child Welfare (MoHCW) in Zimbabwe.

Financial support: This research was funded by the Department for International Development (DFID), United Kingdom.

REFERENCES

1. Victora CG, Adair L, Fall C, Hallal PC, Martorell R, Richter L, Sachdev HS (2008). Maternal and Child undernutrition: consequences for adult health and human capital. *Lancet. Jan 26; 371 (9609):* 340--357.
2. Black RE, Allen LH, Bhutta ZA, Caulfield LE,Onis M, Ezzati M, Mathers C, Rivera J (2008). Maternal and child undernutrition: global and regional exposures and health consequences. *Lancet 371:* 243--260.
3. Dewey KG, Adu-Afarwuah S (2008). Systematic review of the efficacy and effectiveness of complementary feeding interventions in developing countries. *Maternal and Child Nutrition 4:* 24--85.
4. Solomons NW, (2003). Environmental contamination and chronic inflammation influence human growth potential. *J Nutr. 13:* 1237.
5. Black RE, Morris SS, Bryce J (2003). Where and why are 10 million children dying every year? *Lancet 361:*2226--2234.
6. Briend A, Hasan KZ, Aziz KMA, Hoque BA (1989). Are diarrhoea control programmes likely to reduce childhood malnutrition observations from rural Bangladesh*? Lancet 2:*319--322.
7. Humphrey JH (2009).Child undernutrition, tropical enteropathy, toilets, and hand washing. *Lancet 374:* 1032--1035
8. Lunn PG, Northrop-Clewes CA, Downes RM (1991). Intestinal permeability, mucosal injury and growth faltering in Gambian infants. *Lancet 338:* 907--910.
9. Wagner EG, Lanoix J, 1958. Excreta disposal for rural areas and small countries. *WHO monograph series: WHO: Geneva, No. 39.*

10. Curtis V, Cairncross S, Yonli R (2000). Domestic hygiene and diarrhea – pinpointing the problem. *Tropical Medicine and International Health.* 5 **(1)**; 22–32.
11. Marquis GS, Ventura G, Gilman RH,Porras E, Miranda E, Carbajal L, Pentafiel M (1990). Fecal contamination of shanty town toddlers in households with non-corralled poultry, Lima, Peru. *American journal of public health 80 (2):* 146--149.
12. Young SL, Sherman PW, Lucks JB, Pelto GH (2011). Why on earth? Evaluating hypothesis about the physiological functions of human geophagy. *The quarterly review of biology 86 (2):* 97--120.
13. Kabuki DY, Kuaye AY, Wiedmann M, Boor KJ (2004). Molecular subtyping and tracking of Listeria monocytogenes in Latin-style fresh-cheese processing plants. *J. Dairy Sci. 87:*2803--2812.
14. Kung'u JK, Boor KJ, Ame SM, Ali NS, Jackson AE, Stoltzfus RJ (2009). Bacterial populations in complementary foods and drinking-water in households with children aged 10-15 months in Zanzibar, Tanzania. *J. Health Pop. Nutr. 27:* 41--52.
15. Halder AK, Tronchet C, Akhter S,Bhuiya A, Johnston R, Luby SP (2010). Observed hand cleanliness and other measures of hand washing behaviour in rural Bangladesh. *BMC Public Health 10:*545.
16. Luby SP, Halder AK, Tronchet C,Akhtar S, Bhuiya A, Johnston R (2009). Household characteristics associated with hand washing with soap in rural Bangladesh. *Am. J. Trop. Med. Hyg. 81 (5):* 882--887.

17. Curtis VA, Danquah LO, Aunger RV (2009). Planned, motivated and habitual hygiene behaviour: an eleven country review.*doi: The Author(s).10.1093/her/cyp002:* 655-673.
18. Curtis V, Schmidt W, Luby S, Florez R, Touré O, Biran A (2011). Hygiene: new hopes, new horizons. *Lancet Infect Dis*; 11: 312–21
19. Iroegbu CU, Ene-Obong HN, Uwaegbute AC, Amazigo UV (2000). Bacteriological quality of weaning food and drinking water given to children of market women in Nigeria: implications for control of diarrhoea. *J Health PopulNutr.18:*157--62.
20. Afifi ZE, Nasser SS, Shalaby S, Atlam SA (1998). Contamination of weaning foods: organisms, channels, and sequelae. *J Trop Pediatr. 44:*335--337.
21. Pickering AJ, Davis J, Walters SP, Horak HM, Keymer DP, Mushi D , Strickfaden R,Chynoweth JS,Liu J, Blum A,RogersK, Boehm AB (2010). Hands, water and health: Fecal contamination in Tanzanian communities with improved non-networked water supplies. *Envrion. Sci.Technol. 40:* 3267--3272.
22. Pickering AJ, Julian TR, Mamuya S, Boehm AB, Davis J (2011). Bacterial hand contamination among Tanzanian mothers varies temporally and following household activities. *Trop. Med. Int. Health*, 16 (2), 233−239

CHAPTER 3

PREDICTORS OF POOR NUTRITIONAL STATUS IN LESS THAN 5 YEAR OLD ETHIOPIAN CHILDREN- AN EMPHASIS ON WATER, SANITATION AND HYGIENE (WASH)

ABSTRACT

The immediate causes of malnutrition in early childhood are inadequate nutrient intake and frequent infections. Of these causes, nutrition has received considerable attention and efficacious diet interventions are able to reduce child growth deficit by one third. Water, sanitation and hygiene (WASH) interventions are primary interventions for many childhood infections but their role in preventing childhood malnutrition is not well studied, especially in relation to infant feeding. In a cross sectional data analysis of *Alive and Thrive Initiative* baseline data (n=2,992) from two regions in Ethiopia (SNNPR and Tigray), a personal and household environmental hygiene index was the most important predictor of linear growth faltering in 2-5 years old children. A unit increase in the 8-point hygiene scale (dirtier household) was associated with 0.06 decrease in height-for-age Z (HAZ, $p < 0.05$). The association was independent of infant feeding variables. In multivariate models, children from the dirtiest households had 0.5 Z score lower HAZ than children from the cleanest households. A striking reverse association was found between breast feeding and HAZ for children 12-23.9 months ($\beta = -1.51$ Z, $p<0.001$) and 24-59 months old ($\beta = -0.77$ Z, $p<0.001$), which is attributed to reverse causality whereby poor growth leads to breast

feeding and not the reverse. Our results point to the need for a more rigorous approach to hygiene interventions to combat stunting.

INTRODUCTION

Childhood undernutrition, a risk factor for diminished child survival and health, remains prevalent in low and middle income. Most under nutrition begins during pregnancy (intrauterine growth restriction) and the first two years of life leading to higher infant mortality rates, stunting, low birth weight and premature delivery. Stunting, severe wasting and intrauterine growth restriction are together responsible for 2.2 million deaths and 21% of disability-adjusted life-years (DALYS) in children younger than 5 years, the largest percentage of any risk factor in this age group (1).

Stunting, an indicator of chronic malnutrition, has long term consequences which include shorter adult height, reduced school outcome, reduced productivity and income earning capacity in adults, and underweight off spring (2). Evidence suggests that younger children are more responsive to interventions to prevent or treat stunting (3). Preventing stunting during the critical window of opportunity, pregnancy to 24 months, and (4) is critical to future human capital development (2). With almost half of children less than 5 years old stunted (5), Ethiopia is faced with a great challenge to meet many of the Millennium Development Goals (MDGs).

While poor diet is a primary cause of stunting, it is not the only cause. Poor sanitation and hygiene in low income countries also contributes to poor growth in children by causing infectious diseases like diarrhea, which may affect biological utilization of food, a key aspect of food and nutrition security. The inter-relationships between

sanitation, hygiene and dietary causes of stunting are poorly understood because Infant and Young Child Feeding (IYCF) practices have generally been evaluated separately from WASH practices.

There are few studies on improved water supply or sanitation and child growth, and these have reported mixed findings. Among less than 5 year old in rural Lesotho, children from homes that had latrines had on average 0.27 higher height for age Z score (HAZ) than those from homes without latrines (after adjusting for age, sex, water availability, hygiene habits and other socioeconomic variables (6). Presence of water and sanitation facilities was positively associated with HAZ among 1-5 y old from low socioeconomic groups in urban and rural Philippines (7). However, in the same population an inverse association was reported for water and sanitation facilities and risk of stunting in children over 6 months of age, in a prospective study (8). In Sudan, a 20% lower chance of stunted children recovering was reported if they came from homes without water supply, compared to those having water in Khartoum and Crezira regions (9). Incremental improvement in sanitation was associated with incremental improvements in HAZ in a cross sectional data analysis of DHS data from 8 countries (10). The magnitude of the effect of sanitation on growth improved with better water sources.

The effects of water and sanitation on child growth are complex and many and may involve interaction. A synergistic effect of water and sanitation on growth was reported among infants in Lesotho in a prospective study (11) but a similar synergy was not found in Sudan (12). The lack of a synergistic effect in the study by Merchant *et al*, (12) could be due to modification by the condition of the external environment, by hygienic practices or dietary practices. Most studies on water and sanitation have focused

on clinical diarrhea as the outcome of study and even the studies having growth as outcome have not examined the role of environmental hygiene, i.e. indoor and outdoor household environment cleanliness.

This study deviates from the past studies by systematically constructing sanitation and hygiene variables from a detailed survey including both reported and observed indicators. It provides a missing link on the role of personal and environmental hygiene in predicting poor growth outcomes. Most importantly, the study explores the role of WASH in the context of infant and young child feeding and points to the critical role of assessing the two domains in combination when looking at child growth as an outcome. To our knowledge, this is the first study that systematically examines WASH relationship with growth outcomes and how these relationships are influence by age and IYCF practices.

This systematic assessment of evidence on WASH is critical at a time when multifaceted approaches to alleviating growth faltering and child survival are being advocated. With the recent hypothesis on environmental enteropathy as the potential major pathway associated with stunting (13), a holistic approach in studying the infant's environment is critical to inform the design of future programs targeting the critical one thousand-days window of opportunity in preventing or reversing growth retardation.

Research Questions

This study was conducted to assess the relationship between WASH and IYCF indicators as predictors of stunting and wasting in Ethiopia. We answered the following questions 1) What is the prevailing status of WASH and IYCF practices in two regions in Ethiopia (SNNPR and Tigray)? 2) What are the relationships between WASH and

stunting and wasting in young children? 3) How do WASH-nutrition outcome relationships vary after adjusting for IYCF? 4) How do WASH-nutrition or WASH-IYCF-nutrition relationships vary by household food security? 5) How do these relationships vary by child characteristics such as age, sex, illness? The relationships are presented in the conceptual framework, **Figure 3.1**.

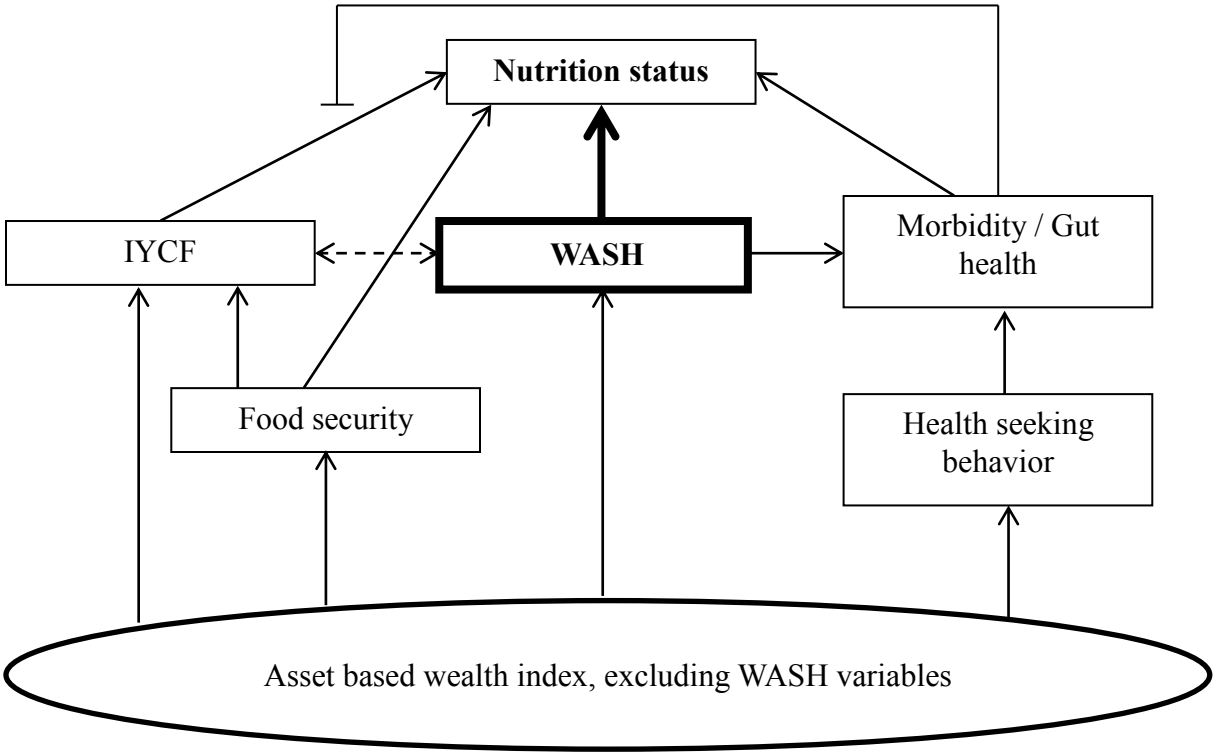

Figure 3.1: The conceptual framework

SUBJECTS AND METHODS

Alive and Thrive Initiative Baseline Data

This study was conducted using baseline data from a cross-sectional survey conducted as part of impact evaluation of Alive and Thrive's community based intervention in SNNPR and Tigray regions, in Ethiopia. The baseline survey

commenced in June of 2010 preceding the Alive and Thrive large scale IYCF practices promotion to prevent stunting. The A&T's community based intervention is implemented through a partner organization called Integrated Family Health Program (IFHP). The data survey was conducted by Addis Continental Institute of Public Health (ACIPH), a national institute with experience in both teaching and conducting research, contracted by IFPRI, who conducted the evaluation research for Alive and Thrive.

Sampling of households and respondents

A total of 3000 children between 0 to 59.9 months were selected using two-stage cluster sampling method. The sample size was determined to detect a 18 percent reduction of stunting in this age at the end of the project, with 90% power and two-sided test and a significance level of α=0.05, and adjusting for correlations within *Woredas* (districts). The primary sampling unit (PSU) or the first cluster was the rural enumeration areas (EA) from the IFHP rural *woredas* in the two regions (14). EAs are geographical units devised by the Central Statistical Authority (CSA) which consists of 150-200 households. The EAs were selected using probability proportion to size (PPS) in relation to population of the EAs after listing all the EAs in the intervention *woredas*.

Using the program CSA uses for Demographic Health Survey (DHS) (15), 75 EAs from the targeted *woredas* were selected. In the second stage, a complete household listing with the number of children residing in each household in each selected cluster was developed. From the household list, three separate sampling frames, for each age group (0-5.9 months, 6-23.9 months, and 24-59.9 months) were created. One index child per household was chosen. From each EA, a total of 40

households with children in the target age categories, were systematically selected from the household list using sampling interval (total number of children/ total number of target population). Out of the 40 households in each EA, eight households for the 0-5.9 m category, 12 households for the 6-23.9 m category and rest in the 24-59 m category in order to meet the target sample size. If the required number was not met in some of the EAs, especially in the lowest category, it was compensated from other selected study sites.

Household questionnaire

The household questionnaire included modules on causes of malnutrition and nutrition outcomes as well as factors that could influence the uptake and adoption of Alive and Thrive interventions, such as household food security, socioeconomic status, parental characteristics, maternal knowledge and skills about IYCF, exposure to Alive and Thrive and other IYCF/ nutrition interventions, exposure to media, household gender relationships, child characteristics e.g., age, gender, perceptions about size and birth. The basic theory for the survey was drawn from the UNICEF conceptual framework on pathway to reduction of Malnutrition (UNICEF, 1997). The questions were largely based on previously validated questions or modules incorporating local norms and terms from formative research in SNNPR and Tigray. The final questionnaire was translated into Amharic and pretested before administering at the household level. The respondent for the household was the mother or immediate caregiver of the child. Of the 3,000 households selected, 2992 (99.7%) mothers responded to the survey.

Data Analysis

Multivariate regression analysis was carried out for HAZ and WHZ as the dependent variables. The analyses were done separately for each age group: 0-5.9, 6-11.9, 12-23.9 and 24-59.9 months old. The independent variables (predictors) were fitted in the models as follows: water source type (improved = 0, non-improved = 1), time to fetch water and back (> 20 minutes = 1, 0 ≥ 20 minutes = 0), type of toilet used by young children (improved = 0, non-improved = 1), animal feces observed (no = 0, yes = 1) and human feces observed (no = 0, yes = 1). Piped water into dwelling place or compound, piped water outside the compound, covered well, protected spring were classified as improved water sources, whereas open well, open spring, river, pond/lake/dam, rain water and others (but not protected sources) were classified as non-improved. Household own pit latrine or flush toilet, traditional pit toilet or ventilated improved pit latrine (VIP) were classified as improved toilet while non-improved were households with either no toilet facility (used the bush or field), open pit, or shared with a neighbor and other practices that did not specify way of fecal disposal or where fecal contact was likely.

A hygiene index was created as an additive score of various hygiene attributes, where each negative attribute was given a score of one for: mother's hands cleanliness, child's hands cleanliness, compound appearance relative to hygiene, area around house needed to be swept, garbage around the house/compound was observed, general appearance inside the house, the floor inside the house needed to be swept and a pile of dirty clothes observed. The corresponding positive attribute was given a score of 0 for each of the item included in the hygiene index. Internal validity of the hygiene index

was evaluated by calculating the Cronbach α coefficient (16) which was equal to 0.77 and satisfactory, i.e. >0.7 (17). Similarly indices for water and sanitation variables were constructed, but the internal validity was too low <0.7, and therefore individual items of the indices were used in the regression models.

Each positive infant feeding practice was scored one (zero for the contrary). The scores for each IYCF indicator category are shown in Table 3.1.

Table 3.1: Scores for IYCF indicators for each age group

	Age group (months)			
IYCF variable	0-5.9	6-11.9	12-23.9	24-59.9
Initiation of breastfeeding (<1 hour)	Yes=1,No=0	Yes = 1, No=0	Yes= 1, No=0	_
Exclusive breast feeding	Yes= 1, No=0	_	_	_
Bottle feeding	Yes = 0,No=1	_	_	_
Continued breast feeding	_	Yes =1, No = 0	Yes=1, No=0	Yes=1, No=0
Animal protein	_	Yes = 1, No = 0	Yes=1, No=0	Yes=1, No=0
Diet diversity*	_	"0" = 0, "1-3"= 1, "4+" =2	"0" = 0, "1-3"= 1, "4+" =2	"0" = 0, "1-3"= 1, "4+" =2
Meal frequency#	_	"0" = 0, "1-2" = 1, "3+" = 2	"0-1" = 0, "2-3"=1, "4+" = 2	"0-2 = 0, "3-4"=1, "5+" = 2

*Diet diversity scores were given for categories of number of different food groups fed to the child in the previous 24 hours (18).
Meal frequency scores were given for categories of number of meals fed to the index child in the previous 24 hours (adapted from reference 19).

The multivariate regression analyses with HAZ as the outcome of interest were adjusted for mother's age and height, child's sex, age and size at birth. Other covariates included number of morbidity symptoms (as an additive score of diarrhea, fever and short breath in the past two weeks), number of under 5 year olds in the households, food security status, socio-economic status (SES) of the households and region. Regression models with WHZ as the outcome variable included mother's BMI as a categorical variable; BMI (underweight <18.5, normal 18.5-24.9 and overweight 25-29.9 kg/m^2) in

addition to the other covariates aforementioned for HAZ. Other covariates considered but not included in the final models were marital status and spouse level of education.

The asset based SES score was constructed using principal component analysis while excluding WASH related assets; toilet, water sources and water treatment. The assets included in the SES score were, namely; ownership of residence, electricity, type of fuel used for cooking, main floor material, number of radios available in the household, number of mobile phones available, number of leather beds, number of jewelry, number of modern chairs, number of modern tables, number of cows and oxen, number of goats and sheep, number of chicken or ducks, number of horses, donkeys or mules and number of hectares of cultivated land.

Three models were fitted for each of the outcome variable. The first model (WASH model) included all WASH indicators (Water source type, time to fetch water and back, presence of animal feces, presence of human feces, type of toilet used by young children and hygiene index) and all the covariates. The second model (IYCF model) included IYCF indicators and all covariates. The third model (Full model) had all the WASH and IYCF variables and all covariates included.

RESULTS

Maternal, child and household characteristics

A majority of the mothers (90%) were over 20 years of age and two thirds of all the mothers (65%) had never attended school. 25% of the mothers were underweight (BMI ≤ 18.5), **Table 3.2**. 50% of the index children were ≥ 24 months old, 29%; 6-23.9

months and 20% were ≤ 6 months old. 52 percent of the index children were male and the rest were female. The mean (SD) child age was 24 (17) months.

Nearly half of the households (42%) had non-improved water sources, took 20 minutes or more to the water source and back (44%) or had non-improved toilet used by children (47%). Generally, households from Tigray had significantly poorer WASH conditions compared to SNPPR, except for non-improved water sources, where 45% of households in SNNPR had non-improved water sources compared to 37% in Tigray ($p<0.001$). More water sources in Tigray (55%) would take 20 minute or more to get to and back to the households compared to 39% in SNNPR ($P < 0.001$). Tigray region had a higher percentage of households with non-improved toilets used by children (53%) compared to SNNPR (44%, $P < 0.00$)1. More striking, Tigray had 59% of the households with animal feces in the compound during time of the survey visit, which was twice the percentage of households with animal feces in SNNPR (24%, $p<0.001$). Despite the poor WASH conditions in Tigray, a significantly greater proportion of children ≤ 5 years (64%) had no diarrhea, fever or chest congestion symptoms self-reported by mothers two weeks prior to the survey, compared to SNNPR (55%, $p<0.001$). Based on the non-WASH assets SES score, Tigray region had better off households (44% in highest tertile) compared to SNNPR (27%, $p < 0.001$).

Table 3.2: Child, maternal and household characteristics in two regions in Ethiopia, June 2010

Characteristics	Region						
	Tigray		SNNPR		Total		p value
	n	Percent/mean±SD	N	Percent/mean±SD	n	Percent/mean±SD	
Child							
Child age in months	1,031	24.4±16.7	1,931	24.4±17.2	2,962	24.4±17.0	0.961
Sex of child							
Male	550	53.4	975	50.5	1,525	51.5	0.135
Female	480	46.6	955	49.5	1,435	48.4	
Birth size							
Bigger than average	154	17.09	681	37.0	835	30.4	0.000
Average	401	44.5	652	35.4	1,053	38.4	
Smaller than average	346	38.4	506	27.5	852	31.0	
Number of morbidity symptoms							
None	656	63.6	1,066	55.2	1,722	58.1	0.000
Greater or equal to 1	375	36.4	865	44.8	1,240	41.8	
Prevalence of stunting							
Not stunted	523	53.0	1,050	56.9	1573	55.5	0.047
Stunted (HAZ < - 2)	463	47.0	794	43.0	1257	44.4	
Prevalence of wasting							
Not wasted	908	90.8	1,766	94.6	2674	93.3	0.000
Wasted (WHZ < -2)	92	9.2	99	5.3	191	6.6	
Prevalence of underweight							
Not under underweight	721	70.5	1513	79.3	2234	76.2	0.000
Underweight (WAZ < -2)	302	29.5	395	20.7	697	23.7	
HAZ	986	-1.78±1.49	1,844	-1.64±1.73	2,830	-1.69±1.65	0.035
WHZ	1000	-0.55±1.20	1,865	-0.05±1.25	2,865	-0.23±1.26	0.000
WAZ	1023	-1.40±1.20	1,908	-0.97±1.33	2,931	-1.12±1.30	0.000

Table 3.2: Child, maternal and household characteristics in two regions in Ethiopia, June 2010

Characteristics	Region						
	Tigray		SNNPR		Total		p value
	n	Percent/mean±SD	N	Percent/mean±SD	n	Percent/mean±SD	
Maternal							
Mother's age in years	1025	30.4±6.9	1,930	28.5±6.0	2,955	29.1±6.4	0.000
Mother's height (cm)	1017	156.5±8.5	1,925	156.6±8.6	2,942	156.5±8.6	0.769
Mother's education (highest grade completed)							
Never attended	725	71.4	1,194	62.2	1,919	65.4	0.000
Grade 1 and above	290	28.6	726	37.8	1,016	34.6	
Mother's BMI category							
Under weight (< 18.5)	328	32.4	390	20.3	718	24.5	0.000
Normal (18.5-24.9)	678	66.9	1,476	77.0	2,154	73.5	
Over weight (25-29.9)	7	0.7	52	2.7	59	2.0	
Number of prenatal visits during last pregnancy							
1-3 visit	476	59.1	708	62.9	1,184	61.3	0.089
≥ 4 visit	330	40.9	418	37.1	748	38.7	
Household							
WASH							
Water source type							
Improved	654	63.5	1,059	54.8	1,713	57.8	0.000
Non-improved	376	36.5	872	45.2	1,248	42.2	
Time to fetch water and back							
≤ 20 min	462	45.0	1,181	61.3	1,643	55.6	0.000
> 20 min	564	55.0	746	38.7	1,310	44.4	
Type of toilet used by children							

Table 3.2: Child, maternal and household characteristics in two regions in Ethiopia, June 2010

Characteristics	Region						
	Tigray		SNNPR		Total		p value
	n	Percent/mean±SD	N	Percent/mean±SD	n	Percent/mean±SD	
Improved	483	47.0	1,075	55.7	1,558	52.7	0.000
Non-improved	545	53.0	854	44.3	1,399	47.3	
Human feces seen in compound							
No	840	83.4	1,701	88.3	2,541	86.6	0.000
Yes	167	16.6	225	11.7	392	13.4	
Animal feces seen in compound							
No	423	41.4	1,456	75.7	1,879	63.8	0.000
Yes	599	58.6	468	24.3	1,067	36.2	
Hygiene Index (range: 1-8)	591	3.9±2.3	1,250	3.4±2.5	1,841	3.5±2.5	0.000
Other							
Number of under 5 year old	1031	1.4±0.6	1,931	1.5±0.6	2,962	1.5±0.6	0.578
Food security category							
Mildly food insecure and food secure	534	51.8	973	50.4	1,507	51.0	0.466
Moderately and severely food insecure	497	48.2	958	49.6	1,455	49.1	
Socio-economic status							
Lower	329	31.9	814	42.1	1,143	38.6	0.000
Middle	248	24.1	589	30.5	837	28.3	
Higher	454	44.0	528	27.3	982	33.2	

Infant feeding in Tigray and SNNPR

Exclusive breastfeeding (EBF) for the first 6 months was high, 72% of all infants (**Table 3.3**). There was no significant difference in EBF rates between the two regions. Breast feeding rate was high for infants in age groups ≤24 months (99%; 0-5.9 months, 97%; 6-11.9 months, and 92%; 12-23.9 months). Bottle feeding was rare (1%). Thirty three percent of 6-11.9 months old children consumed animal protein in the previous day, with significantly high percentage in SNNPR, 40%, compared to 18% in Tigray ($p = 0.000$). Similarly, 38% of all 12-23.9 months old children consumed animal protein, with a significantly higher percentage in SNNPR. Only 6% of infants 6-23.9 months were fed ≥ 4 food groups, the WHO recommended cut off for minimum diet diversity (18). Diet diversity was also poor for children 24-59 months old. Feeding frequency was also problematic, although not as severely as diet diversity. Overall, half of the infants 6-23.9 months had 3 meals or more in the previous day, as recommended by WHO. A significantly higher percentage of infants 6-23.9 months in SNNPR (52%), were fed at least 3 meals in the previous day compared to 45% in Tigray ($p=0.024$).

Table 3.3: Infant and young child feeding indicators by age group and region

	Region						
	Tigray		SNNPR		Total		
	n	%	n	%	n	%	P value
0-5.9 months							
Early initiation of breastfeeding							
Greater than 1 hour	102	49.5	106	26.5	208	34.3	0.000
0-1 hour	104	50.4	294	73.5	398	65.6	
Exclusive breastfeeding							
No	65	31.5	102	25.5	167	27.5	0.114
Yes	141	68.4	298	74.5	439	72.4	
Breastfeeding							
No	0	0.0	2	0.5	2	0.3	0.309
Yes	206	100.0	398	99.5	604	99.7	
Bottle feeding							
Yes	4	1.9	3	0.7	7	1.1	0.193
No	202	98.0	397	99.2	599	98.8	
6-11.9 months							
Child breast fed in the past 24 hours							
No	5	5.0	4	1.7	9	2.7	0.102
Yes	95	95.0	221	98.2	316	97.2	
Consumption of animal protein							
No	82	82.0	135	60.0	217	66.7	0.000
Yes	18	18.0	90	40.0	108	33.2	
Diet diversity score (number of food groups)							
0	29	29.0	58	25.7	87	26.7	0.211
1-3	70	70.0	156	69.3	226	69.5	
≥4	1	1.0	11	4.8	12	3.6	
Meal frequency score*							
0	37	37.0	81	36.0	118	36.3	0.948
1	16	16.0	34	15.1	50	15.3	
2	47	47.0	110	48.8	157	48.3	
12-23.9 months							
Child breastfed in the past 24 hours							
No	15	7.2	29	8.4	44	8.0	0.613
Yes	192	92.7	314	91.5	506	92.0	
Consumption of animal protein							
No	147	71.0	196	57.1	343	62.3	0.001
Yes	60	28.9	147	42.8	207	37.6	
Diet diversity score (food groups)							
0	6	2.9	13	3.7	19	3.4	0.648
1-3	187	90.3	301	87.7	488	88.7	

Table 3.3: Infant and young child feeding indicators by age group and region

	Region						
	Tigray		SNNPR		Total		
	n	%	n	%	n	%	P value
≥4	14	6.7	29	8.4	43	7.8	
Meal frequency (meals/ day)							
2-3	98	56.6	132	44.9	230	49.2	0.014
≥4	75	43.3	162	55.1	237	50.7	
6-23.9 months							
Child breastfed in the past 24 hours							
No	20	6.5	33	5.8	53	6.0	0.677
Yes	287	93.4	535	94.1	822	93.9	
Consumption of animal protein							
No	229	74.5	331	58.2	560	64.0	0.000
Yes	78	25.4	237	41.7	315	36.0	
Diet diversity score							
0	35	11.4	71	12.5	106	12.1	0.381
1-3	257	83.7	457	80.4	714	81.6	
≥4	15	4.8	40	7.0	55	6.2	
Meal frequency score							
0	37	13.5	81	15.6	118	14.9	0.024
1	114	41.7	166	31.9	280	35.3	
2	122	44.6	272	52.4	394	49.7	
24- 59 months							
Child breastfed in the last 24 hours							
No	423	81.6	604	62.7	1,027	69.3	0.000
Yes	95	18.3	359	37.2	454	30.6	
Consumption of animal protein							
No	365	70.7	561	58.2	926	62.6	0.000
Yes	151	29.2	402	41.7	553	37.3	
Diet diversity score (food groups)							
0	5	0.9	6	0.6	11	0.7	0.764
1-3	468	90.3	874	90.7	1,342	90.6	
≥ 4	45	8.6	83	8.6	128	8.6	
Meal frequency (meals per day)							
0-2	71	13.7	149	15.4	220	14.8	0.483
3-4	285	55.0	537	55.7	822	55.5	
≥ 5	162	31.2	277	28.7	439	29.6	

*meal frequency: number of meals per day, 0 for 6-11.9 m and 0-1 for 12-23.9 m; 1 for 6-9 m, 1-2 for 9-11.9 m, 2-3 for 12-23.9 m; 2 for 6-9 m, ≥3 for 9-11.9 m and ≥4 for 12-23.9 m.

The prevalence of growth faltering in Tigray and SNNPR

The linear growth curve was similar to what has been reported from national representative samples in 54 countries (20). Most linear growth faltering occurred between 6-24 months **(Figure 3.2)**. The prevalence of stunting and wasting was higher in Tigray than SNNP (47% vs 43%, $p<0.05$ and 9% vs 5%, $p < 0.001$), **Figure 3.3**. Children from Tigray had lower HAZ, (mean ± SD; -1.78±1.49) compared to SNNP (mean ± SD; -1.64±1.65, $p<0.05$). Similarly, children from Tigray had lower WHZ (-0.55±1.20) compared to SNNPR (-0.23±1.26), $p<0.001$, Table 3.2. The overall prevalence in our sample (44%) was the same as the national prevalence reported in the recent DHS report (21). Ethiopia has made great strides in reduction of stunting, by 14 percent in the last 11 years, but the prevalence still remains high. The prevalence of wasting in the two regions we studied (7%) was lower than the national prevalence (10%) as of 2011 (21).

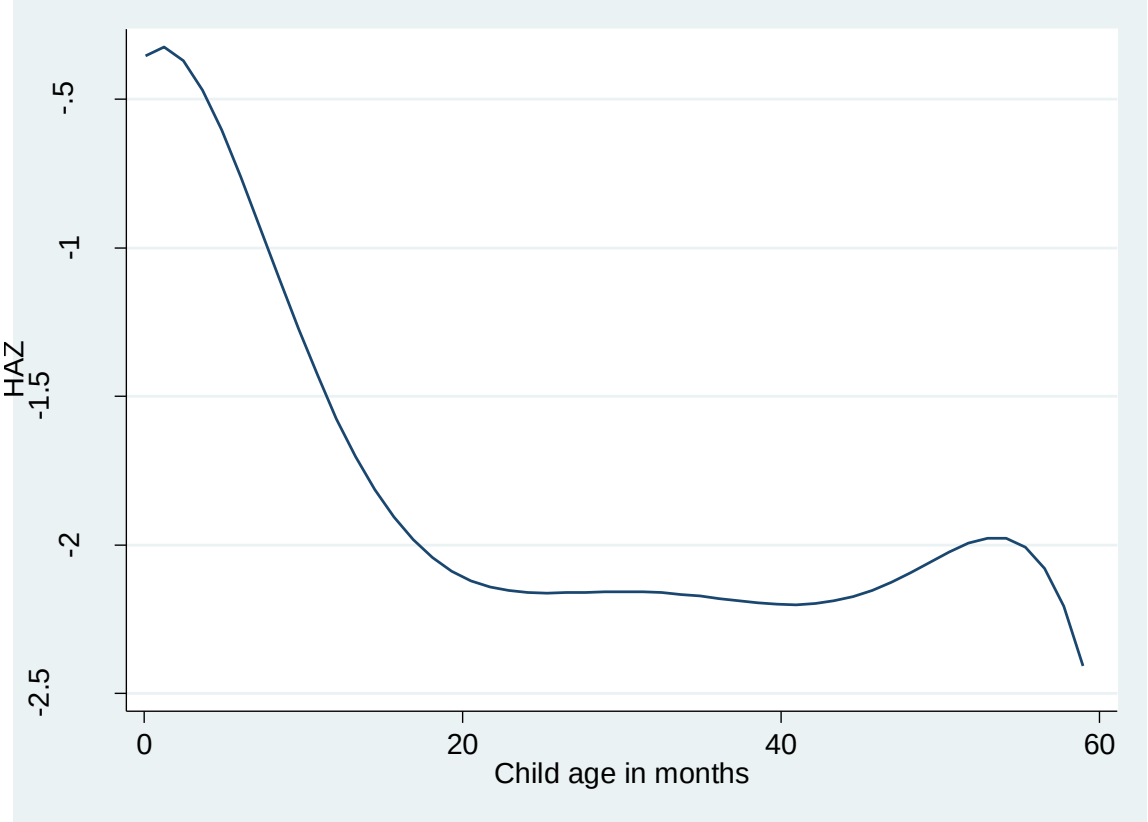

Figure 3.2: Linear growth faltering by age of all children

The plot was derived using STATA 10.0 from raw data by fitting a kernel weighted local polynomial smooth with 6 degrees and 300 as the half width of the smoothing window around each point (n=2,992).

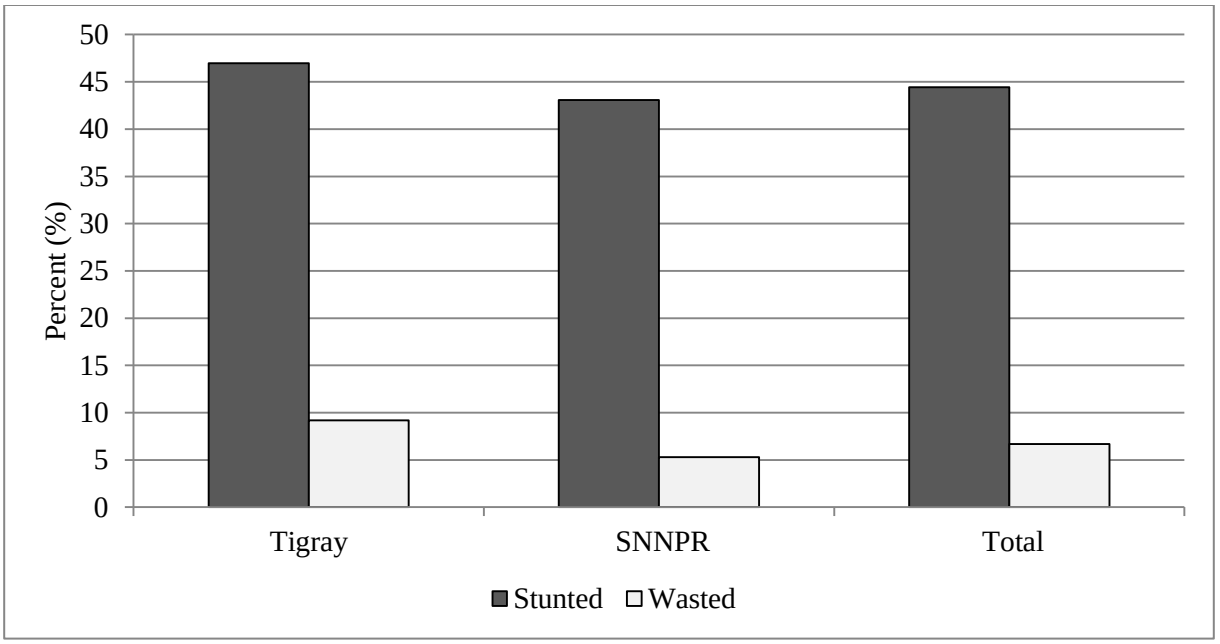

Figure 3.3: Prevalence of stunting and wasting in Tigray and SNNPR

p = 0.047 for the difference in prevalence of stunting by region. P = 0.000 for the difference in prevalence of wasting by region.

Significant predictors of HAZ and WHZ for 0-5.9 months old

No significant associations between WASH indicators and HAZ were observed for this age group. Similarly no significant associations were observed between IYCF indicators and HAZ. The magnitude of coefficients for WASH variables did not change substantially in the model with and without IYCF indicators **(Table 3.4).** Similarly, the coefficients for IYCF did not change remarkably with inclusion of WASH variables. Age of the child (β= -0.24, $p<0.05$), birth size (smaller than average: β= -0.82, $p<0.05$) and average: β= -0.79, $p<0.05$) were associated with lower HAZ whereas mother's height (meters): β= 0.03, $P < 0.05$ was associated with higher HAZ.

Similarly, none of the WASH variables were significant predictors of WHZ. A marginal association is reported for hygiene index and lower WHZ (p =0.085). A 0.44 Z

score difference was observed between adjusted mean WHZ of infants from the dirtiest and cleanest households **(Table 3.8).** Infants from SNNPR had significantly higher WHZ than those from Tigray ($\beta = 0.62$, $p < 0.05$).

Table 3.4: Regression coefficients of the WASH[1], IYCF[2] and Full[3] Models (0-5.9 months)

	HAZ			WHZ[4]		
Variables	WASH	IYCF	Full	WASH	IYCF	Full
Water source type	0.38	_	0.36	0.37	_	0.39
	[-0.19,0.94]		[-0.21,0.94]	[-0.14,0.87]		[-0.12,0.90]
Time to fetch water	-0.18	_	-0.15	0.04	_	0.020
	[-0.74,0.37]		[-0.72,0.42]	[-0.46,0.53]		[-0.48,0.52]
Toilet used by children	-0.18	_	-0.16	-0.08	_	-0.09
	[-0.73,0.38]		[-0.74,0.41]	[-0.59,0.41]		[-0.59,0.42]
Human feces seen	-0.27	_	-0.28	0.12	_	0.15
	[-1.14,0.61]		[-1.16,0.60]	[-0.67,0.91]		[-0.64,0.95]
Animal feces seen	0.54	_	0.48	0	_	-0.01
	[-0.24,1.32]		[-0.30,1.27]	[-0.70,0.70]		[-0.72,0.69]
Hygiene index	0.11	_	0.12	-0.11*	_	-0.11*
	[-0.03,0.25]		[-0.03,0.26]	[-0.23,0.02]		[-0.24,0.02]
Initiation of breastfeeding (within 1 hour)	_	0.27	0.20	_	-0.082	-0.17
		[-0.19,0.73]	[-0.42,0.83]		[-0.52,0.36]	[-0.73,0.38]
Exclusively breastfed	_	-0.36	-0.41	_	0.10	-0.09
		[-0.86,0.14]	[-1.08,0.25]		[-0.36,0.56]	[-0.66,0.49]
Bottle fed (yes)	_	-0.14	-0.07	_	1.07	1.10
		[-1.91,1.64]	[-2.01,1.87]		[-0.64,2.78]	[-0.65,2.85]
N	196	306	196	200	312	200
R^2	0.134	0.074	0.143	0.130	0.079	0.140

95% confidence intervals in brackets, * $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

[1]HAZ = β_0 + β_1Water source + β_2Time to fetch water+ β_3Type of toilet + β_4Presence of human feces + β_5Presence of animal feces + β_6Hygiene index + β_7Child age (months) + β_8Child sex + β_9Birth size + β_{10}Mother's education + β_{11}Mother's age + β_{12}Mother's height + β_{13}Number of under 5-y + β_{14}Morbidity + β_{15}Number of prenatal visits + β_{16}Food security + β_{17}SES + β_{18}Region, R Squared =0.134

Table 3.4 (Continued)

[2]HAZ = β_0 + β_1Initiation of breastfeeding + β_2Exclusive breastfeeding + β_3Bottle feeding + β_4Child age (months) + β_5Child sex + β_6Birth size + β_7Mother's education + β_8Mother's age + β_9Mother's height + β_{10}Number of under 5-y + β_{11}Morbidity + β_{12}Number of prenatal visits + β_{13}Food security + β_{14}SES + β_{15}Region, R Squared =0.074

[3]HAZ = β_0 + β_1Water source + β_2Time to fetch water+ β_3Type of toilet + β_4Presence of human feces + β_5Presence of animal feces + β_6Hygiene index + β_7Initiation + β_8Exclusive breastfeeding + β_9Bottle feeding + β_{10}Child age (months) + β_{11}Child sex + β_{12}Birth size + β_{13}Mother's education + β_{14}Mother's age + β_{15}Mother's height + β_{16}Number of under 5-y + β_{17}Morbidity + β_{18}Number of prenatal visits + β_{19}Food security + β_{20}SES + β_{21}Region, R Squared =0.143

[4]Each of the three models with WHZ as the outcome for 0-5.9 months old were the same as the WASH, IYCF and Full models with HAZ as the outcome except for an additional covariate; mother's BMI

Significant predictors of HAZ and WHZ for 6-11.9 months old

Similar to age group 0-5.9 months, none of the WASH variables was significantly associated with HAZ. The coefficients for the WASH variables did not change substantially when IYCF variables were included in the model **(Table 3.5),** suggesting they were independent of IYCF. Intake of 4 or more food groups within the last 24 hours was associated with 2.93 higher HAZ ($p < 0.05$). The high coefficient could be an unstable estimate due to the small proportion of children in this category 3.7% (12/325). The magnitude of the coefficient for 4+ food groups changed from 1.18 ($p=0.116$), n=17, for IYCF model to 2.93 ($p=0.042$), n=117 for the full model suggesting a correlation between diet diversity and WASH.

Most of the WASH variables had negative coefficients, except animal feces ($\beta = 0.40$, $p=0.262$) for regression models with WHZ as the outcome of interest. Poor WASH conditions were insignificantly associated with decline in WHZ. However, a significant reduction in WHZ ($\beta = -0.53$, $p<0.05$) with increase in number of symptoms observed in the previous two weeks (morbidity) was observed. Higher scores on the hygiene index (meaning worse hygiene) were associated with lower WHZ ($\beta = -0.10$, $p=0.08$). WASH associations with WHZ were independent of IYCF. Initiation of breastfeeding within one hour was marginally associated with lower WHZ ($\beta = -0.51$, $p=0.054$). Birth size (average; $\beta = -0.77$, $p < 0.05$ and smaller than average; $\beta = -0.84$, $p < 0.01$), and increase in number of symptoms ($\beta = -0.53$, $p <0.05$) were significantly associated with lower WHZ.

Table 3.5: Regression coefficients of the WASH[1], IYCF[2] and Full[3] models (6-11.9 months old)

	HAZ			WHZ[4]		
	WASH	IYCF	Full	WASH	IYCF	Full
Water source type	-0.01 [-0.67,0.65]	_	0.08 [-0.61,0.77]	-0.21 [-0.71,0.29]	_	-0.29 [-0.82,0.23]
Time to fetch water	-0.38 [-1.02,0.26]	_	-0.43 [-1.10,0.23]	-0.28 [-0.75,0.20]	_	-0.34 [-0.83,0.16]
Toilet used by children	0.12 [-0.53,0.78]	_	0.21 [-0.47,0.89]	-0.16 [-0.64,0.33]	_	-0.15 [-0.65,0.36]
Human feces seen	0.72 [-0.22,1.65]	_	0.71 [-0.27,1.69]	-0.36 [-1.08,0.36]	_	-0.19 [-0.96,0.58]
Animal feces seen	0.01 [-0.91,0.92]	_	0.08 [-0.88,1.03]	0.38 [-0.29,1.06]	_	0.40 [-0.31,1.12]
Hygiene index	0.09 [-0.06,0.23]	_	0.09 [-0.05,0.23]	-0.09 [-0.20,0.020]	_	-0.100* [-0.21,0.012]
Initiation of breastfeeding (within 1 hour)	_	-0.23 [-0.75,0.30]	-0.34 [-1.03,0.36]	_	-0.32 [-0.75,0.12]	-0.51* [-1.06,0.03]
Breastfed in the last 24 hours	_	1.77* [-0.20,3.73]	1.18 [-2.44,4.81]	_	-0.23 [-1.61,1.15]	-0.75 [-3.50,1.99]
Diet diversity score						
0 food groups (reference)	_	_	_	_	_	_
1-3 food groups	_	0.70 [-0.30,1.70]	0.80 [-0.46,2.06]	_	-0.24 [-1.06,0.58]	-0.31 [-1.29,0.67]
4 + food groups	_	1.18 [-0.50,2.86]	2.93** [0.11,5.76]	_	-0.43 [-1.79,0.94]	-0.49 [-2.63,1.65]
Meal frequency score						
0-2 meals/day (reference)	_	_	_	_	_	_
2-3 meals/day	_	-0.26 [-1.31,0.79]	0.06 [-1.25,1.37]	_	0.11 [-0.73,0.95]	0.41 [-0.58,1.40]
4 + meals/day	_	-0.14 [-1.13,0.85]	-0.19 [-1.46,1.08]	_	0.31 [-0.50,1.13]	0.35 [-0.64,1.34]

Table 3.5: Regression coefficients of the WASH[1], IYCF[2] and Full[3] models (6-11.9 months old)

	HAZ			WHZ[4]		
	WASH	IYCF	Full	WASH	IYCF	Full
Fed animal protein in the last 24 hours	_	-0.28	-0.26	_	0.0012	-0.075
		[-0.92,0.36]	[-1.09,0.58]		[-0.51,0.51]	[-0.72,0.57]
N	119	175	119	120	178	120
R^2	0.205	0.148	0.262	0.229	0.180	0.276

95% confidence intervals in brackets, * $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

[1]HAZ = β_0 + β_1Water source + β_2Time to fetch water+ β_3Type of toilet + β_4Presence of human feces + β_5Presence of animal feces + β_6Hygiene index + β_7Child age (months) + β_8Child sex + β_9Birth size + β_{10}Mother's education + β_{11}Mother's age + β_{12}Mother's height + β_{13}Number of under 5-y + β_{14}Morbidity + β_{15}Number of prenatal visits + β_{16}Food security + β_{17}SES + β_{18}Region, R Squared =0.205

[2]HAZ = β_0 + β_1Initiation of breastfeeding + β_2Breastfeeding + β_3Diet diversity + β_4Meal frequency + β_5Animal protein + β_6Child age (months) + β_7Child sex + β_8Birth size + β_9Mother's education + β_{10}Mother's age + β_{11}Mother's height + β_{12}Number of under 5-y + β_{13}Morbidity + β_{14}Number of prenatal visits + β_{15}Food security + β_{16}SES + β_{17}Region, R Squared =0.148

[3]HAZ = β_0 + β_1Water source + β_2Time to fetch water+ β_3Type of toilet + β_4Presence of human feces + β_5Presence of animal feces + β_6Hygiene index + β_7Initiation of breastfeeding + β_8Breastfeeding + β_9Diet diversity + β_{10}Meal frequency + β_{11}Animal protein + β_{12}Child age (months) + β_{13}Child sex + β_{14}Birth size + β_{15}Mother's education + β_{16}Mother's age + β_{17}Mother's height + β_{18}Number of under 5-y + β_{19}Morbidity + β_{20}Number of prenatal visits + β_{21}Food security + β_{22}SES + β_{23}Region, R Squared =0.262

[4]Each of the three models with WHZ as the outcome for 6-11.9 months old were the same as the WASH, IYCF and Full models with HAZ as the outcome except for an additional covariate; mother's BMI

Significant predictors of HAZ and WHZ for 12-23.9 months old

Non-improved child toilet type was marginally associated with lower HAZ (β = -0.39, p=0.062). Breastfeeding in the prior 24 hour period was strongly associated with lower HAZ (β= -1.51, p<0.001), likely a case of reverse causality (25). There was no substantial change in coefficient for breastfeeding **(Table 3.6)** when the IYCF model was adjusted for WASH. The variability in diet diversity score was minimal, and therefore it was difficult to draw meaningful comparisons for this age group. One month increase in child age was associated with 0.09 lower HAZ (p<0.01).

Presence of animal feces was marginally associated with higher WHZ (β = 0.53, p=0.061). Other WASH indicators were not associated with WHZ. Some modest changes in WASH coefficients suggest correlation with IYCF variables included in the model (Table 3.5). IYCF indicators were not associated with WHZ. Child age (β = 0.07, p<0.05), mother's height (β = 0.05, p<0.01), mother's BMI (18.5 to 25), β = 0.44, p <0.05 and region (SNNP), β = 0.77, p<0.01) were associated with higher WHZ.

Table 3.6: Regression coefficients of the WASH[1], IYCF[2] and Full[3] models (12-23.9 months old)

	HAZ			WHZ[4]		
	WASH	IYCF	Full	WASH	IYCF	Full
Water source type	-0.23 [-0.61,0.16]	_	-0.08 [-0.48,0.32]	0.09 [-0.25,0.44]	_	0.03 [-0.36,0.41]
Time to fetch water	-0.04 [-0.41,0.34]	_	-0.16 [-0.53,0.22]	-0.17 [-0.51,0.17]	_	-0.10 [-0.46,0.27]
Toilet used by children	-0.25 [-0.64,0.13]	_	-0.39* [-0.80,0.02]	0.09 [-0.26,0.43]	_	0.16 [-0.24,0.56]
Human feces seen	0.20 [-0.34,0.74]	_	0.29 [-0.31,0.90]	-0.07 [-0.55,0.42]	_	-0.13 [-0.72,0.46]
Animal feces seen	-0.13 [-0.64,0.39]	_	-0.44 [-1.00,0.12]	0.31 [-0.15,0.77]	_	0.53* [-0.03,1.08]
Hygiene index	-0.01 [-0.11,0.09]	_	0.04 [-0.07,0.14]	0.01 [-0.08,0.10]	_	-0.01 [-0.11,0.09]
Initiation of breastfeeding (within 1 hour)	_	-0.13 [-0.45,0.19]	-0.08 [-0.50,0.35]	_	0.06 [-0.24,0.36]	0.06 [-0.36,0.49]
Breastfed in the last 24 hours	_	-1.43**** [-2.01,-0.85]	-1.51**** [-2.26,-0.75]	_	0.06 [-0.48,0.61]	0.34 [-0.42,1.11]
Diet diversity score						
0 food groups (reference)	_	_	_	_	_	_
1-3 food groups	_	0 .	0.20 [-0.52,0.93]	_	0.02 [-0.46,0.50]	-0.30 [-1.01,0.40]
4 + food groups	_	-0.10 [-0.61,0.41]	0 .	_	0 .	0 .
Meal frequency (meals/day)						
0-2 (reference)	_	_	_	_	_	_
2-3	_	-0.04 [-0.34,0.27]	0 .	_	0.07 [-0.21,0.35]	0.20 [-0.20,0.59]
≥ 4	_	0 .	-0.06 [-0.46,0.34]	_	0	0 .

Table 3.6: Regression coefficients of the WASH[1], IYCF[2] and Full[3] models (12-23.9 months old)

	HAZ			WHZ[4]		
	WASH	IYCF	Full	WASH	IYCF	Full
Animal protein in the last 24 hours	_	0.074	0.073	_	-0.07	0.17
		[-0.25,0.40]	[-0.34,0.48]		[-0.38,0.23]	[-0.23,0.57]
N	216	295	185	215	294	184
R^2	0.166	0.237	0.268	0.237	0.195	0.273

95% confidence intervals in brackets, * $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

[1]HAZ = β_0 + β_1Water source + β_2Time to fetch water+ β_3Type of toilet + β_4Presence of human feces + β_5Presence of animal feces + β_6Hygiene index + β_7Child age (months) + β_8Child sex + β_9Birth size + β_{10}Mother's education + β_{11}Mother's age + β_{12}Mother's height + β_{13}Number of under 5-y + β_{14}Morbidity + β_{15}Number of prenatal visits + β_{16}Food security + β_{17}SES + β_{18}Region, R Squared =0.166

[2]HAZ = β_0 + β_1Initiation of breastfeeding + β_2Breastfeeding + β_3Diet diversity + β_4Meal frequency + β_5Animal protein + β_6Child age (months) + β_7Child sex + β_8Birth size + β_9Mother's education + β_{10}Mother's age + β_{11}Mother's height + β_{12}Number of under 5-y + β_{13}Morbidity + β_{14}Number of prenatal visits + β_{15}Food security + β_{16}SES + β_{17}Region, R Squared =0.237

[3]HAZ = β_0 + β_1Water source + β_2Time to fetch water+ β_3Type of toilet + β_4Presence of human feces + β_5Presence of animal feces + β_6Hygiene index + β_7Initiation of breastfeeding + β_8Breastfeeding + β_9Diet diversity + β_{10}Meal frequency + β_{11}Animal protein + β_{12}Child age (months) + β_{13}Child sex + β_{14}Birth size + β_{15}Mother's education + β_{16}Mother's age + β_{17}Mother's height + β_{18}Number of under 5-y + β_{19}Morbidity + β_{20}Number of prenatal visits + β_{21}Food security + β_{22}SES + β_{23}Region, R Squared =0.268

[4]Each of the three models with WHZ as the outcome for 12-23.9 months old were the same as the WASH, IYCF and Full models with HAZ as the outcome except for an additional covariate; mother's BMI

Significant predictors of HAZ and WHZ for 24-59.9 months old

A unit increase in hygiene score was associated with 0.06 lower HAZ, $p<0.05$ **(Table 3.7)** while presence of animal feces was associated with a 0.37 higher HAZ ($p <0.05$). The association between hygiene index and HAZ was independent of SES and recent morbidity. The hygiene index coefficient did not change substantially with IYCF indicators included in model suggesting the association with HAZ was independent of IYCF (Table 3.7). The other WASH variables were not associated with HAZ.

Of the IYCF indicators, breastfeeding was significantly associated with 0.77 lower HAZ score ($p <0.001$), likely a case of reverse causality (25). Other predictors associated with lower or higher HAZ were birth size (average: $\beta= -0.33$, $p<0.05$), smaller than average ($\beta= -0.43$, $p< 0.01$), mother's height ($\beta= 0.04$, $p < 0.001$), more than 4 prenatal visits ($\beta= -0.32$, $p < 0.05$) and region (SNNPR), $\beta = 0.40$, $p < 0.01$.

None of the WASH predictors were significantly associated with WHZ in this age group (Table 3.7). A reverse association of breastfeeding and WHZ is also reported for this age group ($\beta = -0.24$, $p < 0.05$). Increase in number of meals was associated with higher WHZ (≥5 meals in a day: $\beta = 0.37$, $p <0.05$). A difference of 0.34 Z score between adjusted mean WHZ of children who received 5 meals or more and those who received 0-2 meals a day is reported ($p=0.012$), **Table 3.10**. Birth size (average: $\beta = -0.38$, $p <0.01$ and smaller than average; $\beta = -0.23$, $p=0.056$) and mother's age, ($\beta = -0.02$, $p < 0.05$) were associated with lower WHZ. Mother's BMI (18.5 to 25), $\beta = 0.52$, $p < 0.001$ and region (SNNPR, $\beta = 0.27$, $p < 0.05$) were associated with higher WHZ.

Table 3.7: Regression coefficients of the WASH[1], IYCF[2] and Full[3] Models (24-59.9 months)

	HAZ			WHZ[4]		
	WASH	IYCF	Full	WASH	IYCF	Full
Water source type	-0.19 [-0.46,0.09]	_	-0.12 [-0.38,0.15]	-0.024 [-0.22,0.18]	_	0.01 [-0.19,0.21]
Time to fetch water	0.08 [-0.17,0.34]	_	0.10 [-0.15,0.35]	0.03 [-0.15,0.22]	_	0.08 [-0.11,0.26]
Toilet used by children	-0.12 [-0.38,0.13]	_	-0.045 [-0.30,0.21]	-0.18* [-0.37,0.01]	_	-0.15 [-0.34,0.04]
Human feces seen	0.17 [-0.18,0.52]	_	0.19 [-0.16,0.53]	-0.013 [-0.27,0.25]	_	0.04 [-0.22,0.30]
Animal feces seen	0.36** [0.05,0.67]	_	0.37** [0.07,0.67]	0.012 [-0.22,0.24]	_	-0.01 [-0.23,0.22]
Hygiene index	-0.07** [-0.13,-0.01]	_	-0.06** [-0.12,-0.003]	-0.012 [-0.06,0.03]	_	-0.01 [-0.06,0.03]
Breastfed in the last 24 hours	_	-0.81**** [-1.04,-0.59]	-0.77**** [-1.05,-0.48]	_	-0.18** [-0.35,-0.01]	-0.24** [-0.46,-0.02]
Diet diversity (food groups)						
0	_	_	_	_	_	_
1-3	_	1.06 [-0.30,2.43]	1.36 [-0.58,3.30]	_	0.36 [-0.57,1.28]	-0.17 [-1.41,1.06]
≥4	_	1.05 [-0.36,2.45]	1.36 [-0.63,3.35]	_	0.51 [-0.46,1.47]	0.00 [-1.27,1.28]
Meal frequency (meals/day)		_	_	_	_	_
0-2						
3-4	_	0.11 [-0.16,0.38]	0.21 [-0.12,0.54]	_	0.21** [0.01,0.41]	0.23* [-0.03,0.48]
≥5	_	-0.097 [-0.40,0.21]	-0.13 [-0.51,0.25]	_	0.20* [-0.04,0.43]	0.37** [0.09,0.66]
Animal protein in the last 24 hours	_	0.12 [-0.10,0.34]	0.19 [-0.10,0.48]	_	-0.03 [-0.20,0.13]	-0.11 [-0.33,0.11]

Table 3.7: Regression coefficients of the WASH[1], IYCF[2] and Full[3] Models (24-59.9 months)

	HAZ			WHZ[4]		
	WASH	IYCF	Full	WASH	IYCF	Full
N	515	870	515	524	880	524
R^2	0.105	0.113	0.171	0.127	0.114	0.150

95% confidence intervals in brackets, * $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

[1]HAZ = β_0 + β_1Water source + β_2Time to fetch water+ β_3Type of toilet + β_4Presence of human feces + β_5Presence of animal feces + β_6Hygiene index + β_7Child age (months) + β_8Child sex + β_9Birth size + β_{10}Mother's education + β_{11}Mother's age + β_{12}Mother's height + β_{13}Number of under 5-y + β_{14}Morbidity + β_{15}Number of prenatal visits + β_{16}Food security + β_{17}SES + β_{18}Region, R Squared =0.105

[2]HAZ = β_0 + β_1Breastfeeding + β_2Diet diversity + β_3Meal frequency + β_4Animal protein + β_5Child age (months) + β_6Child sex + β_7Birth size + β_{10}Mother's education + β_{11}Mother's age + β_{12}Mother's height + β_{13}Number of under 5-y + β_{14}Morbidity + β_{15}Number of prenatal visits + β_{16}Food security + β_{17}SES + β_{18}Region, R Squared =0.113

[3]HAZ = β_0 + β_1Water source + β_2Time to fetch water+ β_3Type of toilet + β_4Presence of human feces + β_5Presence of animal feces + β_6Hygiene index + β_7Breastfeeding + β_8Diet diversity + β_9Meal frequency + β_{10}Animal protein + β_{11}Child age (months) + β_{12}Child sex + β_{13}Birth size + β_{14}Mother's education + β_{15}Mother's age + β_{16}Mother's height + β_{17}Number of under 5-y + β_{18}Morbidity + β_{19}Number of prenatal visits + β_{20}Food security + β_{21}SES + β_{22}Region, R Squared =0.171

[4]Each of the three models with WHZ as the outcome for 24-59.9 months old were the same as the WASH, IYCF and Full models with HAZ as the outcome except for an additional covariate; mother's BMI

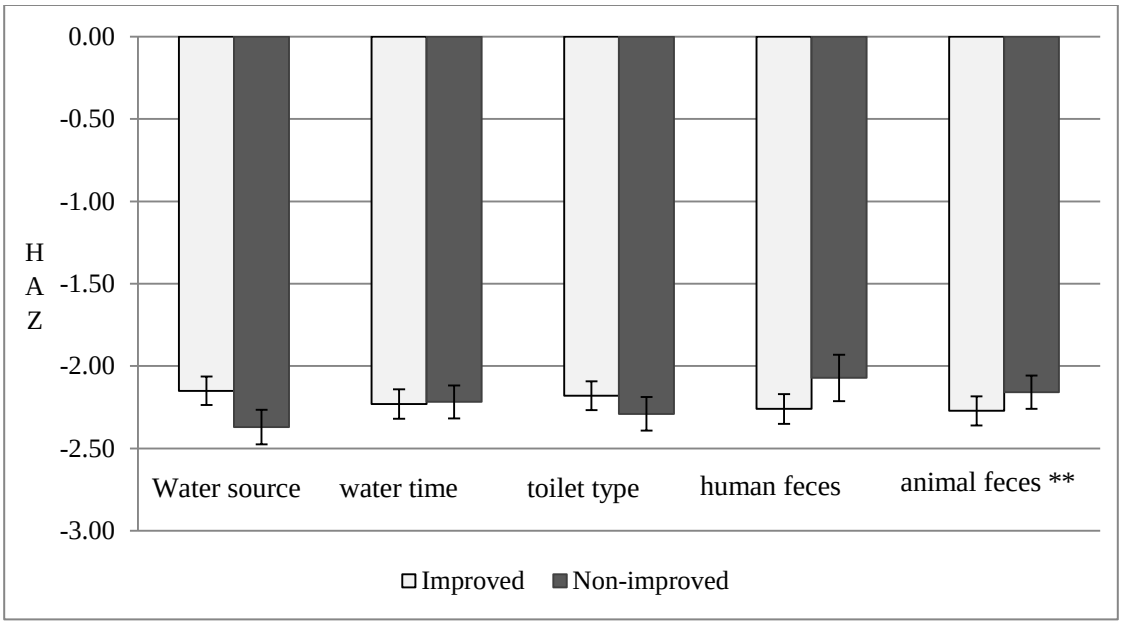

Figure 3.4: Association between water and sanitation variables and Height-for-age Z scores (HAZ) for all 24-59 months old

Adjusted mean HAZ (± SEM), n= 515. Improved: improved water sources, < 20 minutes for water time, improved toilet, no human feces, no animal feces. Non-improved: Non-improved water source, ≥20 minutes for water time, non-improved toilet, yes for human feces, yes for animal feces. Water source; p value = 0.389, water time; p value = 0.418, toilet type used by children; p value = 0.725, human feces; p value = 0.289, **animal feces; p value = 0.015.

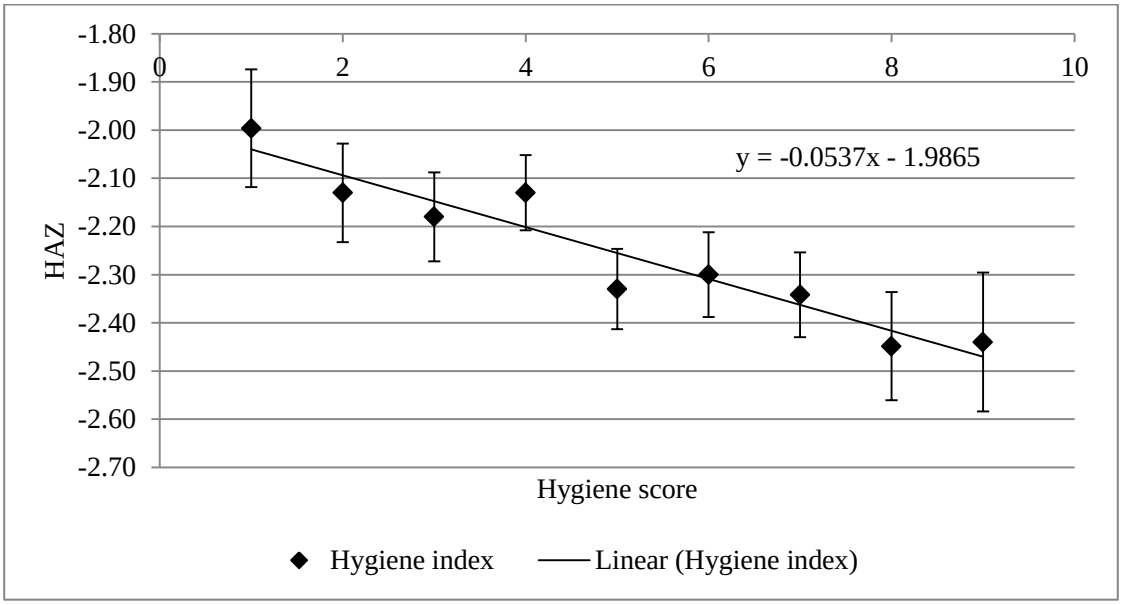

Figure 3.5: Association between hygiene index and Height-for-age Z scores (HAZ) for all 24-59 months old

Adjusted mean HAZ (± SEM), n= 515, p value for the trend = 0.038.

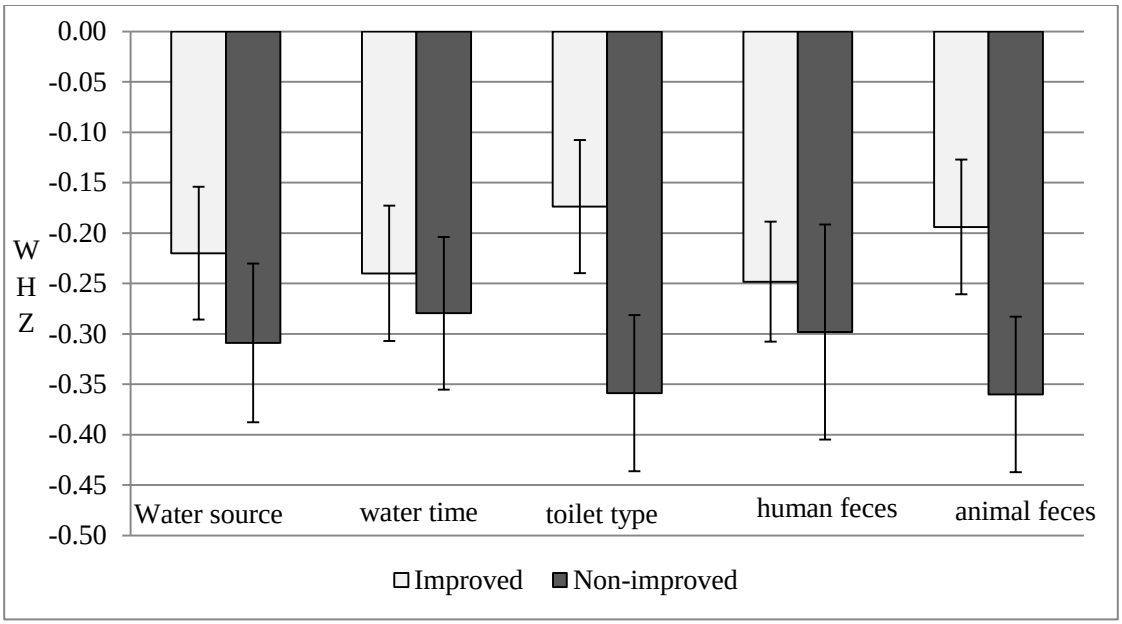

Figure 3.6: Association between water and sanitation variables and weight-for-height Z scores (WHZ) for all 24-59 months old

Adjusted mean WHZ (± SEM), n= 515. Improved: improved water sources, < 20 minutes for water time, improved toilet, no human feces, no animal feces. Non-improved: Non-improved water source, ≥20 minutes for water time, non-improved toilet, yes for human feces, yes for animal feces. Water source; p value = 0.904, water time; p value = 0.433, toilet type used by children; p value = 0.122, human feces; p value = 0.789, animal feces; p value = 0.958.

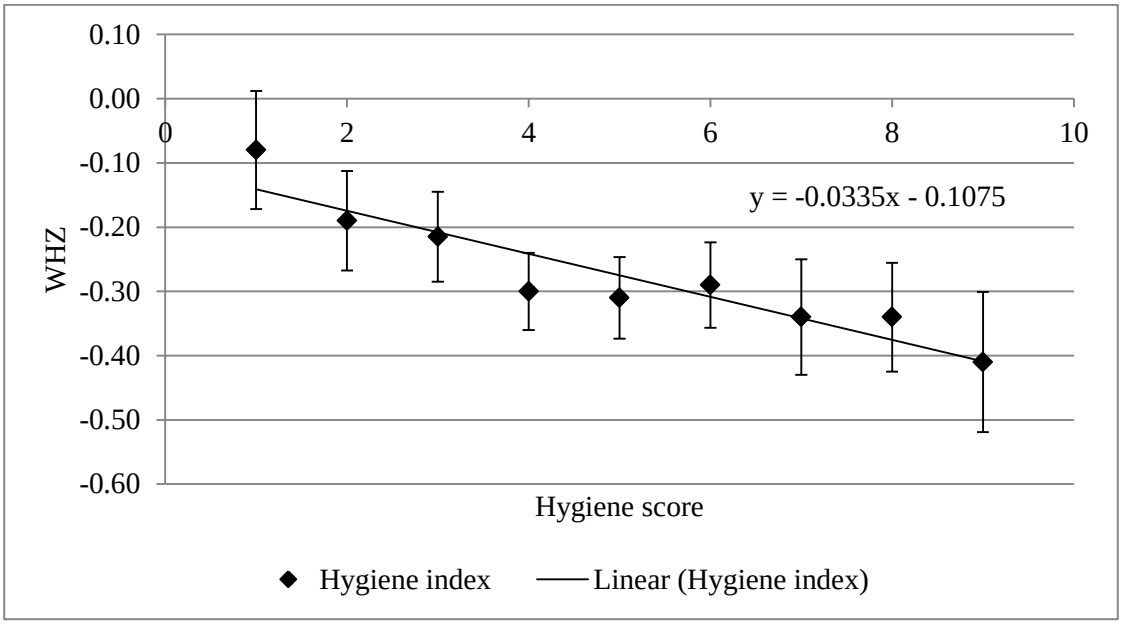

Figure 3.7: Association between hygiene index and weight-for-height Z scores (WHZ) for all 24-59 months old

Adjusted mean WHZ (± SEM), n= 515. P value for the trend = 0.605.

Table 3.8: Adjusted means of HAZ and WHZ for 0-5.9 months old infants

	HAZ				WHZ			
	Mean	SE	95 % CI	P value	Mean	SE	95 % CI	P value
Water source type								
Improved	-0.58	0.17	[-.92 -.24]	0.210	-0.14	0.15	[-.44 .15]	0.135
non-improved	-0.07	0.21	[-.48 .34]		0.11	0.19	[-.25 .48]	
Time to get water and back								
≤ 20 minutes	-0.36	0.19	[-.73 .01]	0.607	0.03	0.17	[-.29 .36]	0.938
> 20 minutes	-0.38	0.19	[-.75 -.01]		-0.12	0.17	[-.45 .21]	
Type of toilet used by children								
Improved	-0.26	0.17	[-.61 .08]	0.571	0.04	0.15	[-.26 .34]	0.740
non-improved	-0.50	0.20	[-.90 -.10]		-0.13	0.18	[-.49 .23]	
Human feces seen								
No	-0.37	0.14	[-.66 -.09]	0.525	-0.01	0.13	[-.26 .24]	0.702
Yes	-0.34	0.33	[-.99 .32]		-0.20	0.30	[-.79 .39]	
Animal feces seen								
No	-0.49	0.16	[-.80 -.17]	0.225	0.08	0.14	[-.20 .36]	0.970
Yes	-0.10	0.23	[-.56 .36]		-0.31	0.21	[-.72 .11]	
Hygiene index								
0	-0.66	0.22	[-1.1 -.23]	0.109	0.30	0.20	[-.09 .69]	0.085
1	-0.76	0.19	[-1.13 -.40]		0.00	0.17	[-.33 .33]	
2	-0.31	0.15	[-.61 -.00]		-0.01	0.14	[-.29 .27]	
3	-0.53	0.21	[-.94 -.12]		-0.05	0.19	[-.42 .32]	
4	0.02	0.22	[-.41 .46]		-0.01	0.20	[-.40 .38]	
5	-0.04	0.22	[-.47 .38]		-0.29	0.19	[-.67 .09]	
6	-0.01	0.26	[-.52 .50]		-0.47	0.23	[-.92 -.02]	
7	-0.18	0.39	[-.95 .59]		-0.59	0.35	[-1.28 .10]	
8	0.78	0.61	[-.42 1.98]		-0.14	0.54	[-1.21 .93]	

Table 3.8: Adjusted means of HAZ and WHZ for 0-5.9 months old infants

	HAZ				WHZ			
	Mean	SE	95 % CI	P value	Mean	SE	95 % CI	P value
Exclusive breastfeeding								
No	-0.33	0.25	[-.82 .16]	0.225	-0.09	0.22	[-.52 .33]	0.765
Yes	-0.38	0.16	[-.69 -.07]		-0.02	0.14	[-.30 .26]	
SES								
Lower	-0.48	0.23	[-.95 -.023]	0.681	-0.04	0.21	[-.45 .37]	0.843
Middle	-0.09	0.25	[-.58 .40]		-0.09	0.22	[-.52 .34]	
Higher	-0.46	0.21	[-.88 -.05]		0.00	0.19	[-.37 .36]	
Food security category								
Mildly insecure or food secure	-0.33	0.18	[-.69 .03]	0.840	0.05	0.16	[-.27 .37]	0.622
Moderate or severely insecure	-0.41	0.19	[-.79 -.04]		-0.14	0.17	[-.47 .20]	
Region								
Tigray	-0.43	0.22	[-.87 .01]	0.651	-0.47	0.20	[-.86 -.08]	0.043
SNNP	-0.34	0.16	[-.66 -.01]		0.19	0.15	[-.10 .48]	

Table 3.9: Adjusted means of HAZ and WHZ for 6-23.9 months old children

	HAZ				WHZ			
	Mean	SE	95 % CI	P value	Mean	SE	95 % CI	P value
Water source type								
Improved	-1.55	0.10	[-1.74 -1.36]	0.863	-0.22	0.09	[-.392 -.06]	0.687
non-improved	-1.62	0.13	[-1.88 -1.36]		-0.37	0.12	[-.59 -.14]	
time to get water and back								
≤20 minutes	-1.46	0.10	[-1.66 -1.25]	0.103	-0.19	0.09	[-.37 -.013]	0.409
> 20 minutes	-1.73	0.12	[-1.97 -1.50]		-0.39	0.11	[-.60 -.18]	
Type of toilet used by children								
Improved	-1.57	0.11	[-1.79 -1.35]	0.473	-0.28	0.10	[-.47 -.09]	0.739
non-improved	-1.58	0.11	[-1.79 -1.36]		-0.27	0.10	[-.46 -.08]	
Human feces seen in compound								
No	-1.62	0.09	[-1.78 -1.45]	0.155	-0.25	0.07	[-.39 -.10]	0.586
Yes	-1.31	0.21	[-1.73 -.89]		-0.46	0.18	[-.82 -.09]	
Animal feces seen in compound								
No	-1.52	0.10	[-1.71 -1.33]	0.428	-0.28	0.08	[-.45 -.12]	0.012
Yes	-1.67	0.14	[-1.94 -1.41]		-0.26	0.12	[-.49 -.03]	
Hygiene index								
0	-1.65	0.14	[-1.92 -1.39]	0.312	-0.19	0.12	[-.42 .05]	0.215
1	-1.48	0.12	[-1.72 -1.24]		-0.16	0.11	[-.37 .05]	
2	-1.60	0.10	[-1.80 -1.40]		-0.32	0.09	[-.50 -.15]	
3	-1.77	0.10	[-1.98 -1.57]		-0.28	0.09	[-.46 -.10]	
4	-1.41	0.11	[-1.62 -1.21]		-0.43	0.09	[-.62 -.25]	
5	-1.55	0.12	[-1.78 -1.32]		-0.15	0.10	[-.35 .05]	
6	-1.56	0.14	[-1.84 -1.28]		-0.49	0.12	[-.73 -.25]	
7	-1.56	0.17	[-1.90 -1.22]		-0.29	0.15	[-.59 .01]	
8	-1.48	0.23	[-1.93 -1.04]		-0.39	0.20	[-.78 .01]	

Table 3.9: Adjusted means of HAZ and WHZ for 6-23.9 months old children

	HAZ				WHZ			
	Mean	SE	95 % CI	P value	Mean	SE	95 % CI	P value
Breastfeeding								
No	-0.89	0.37	[-1.61 -.17]	0.001	-0.11	0.32	[-.74 .52]	0.657
Yes	-1.61	0.08	[-1.77 -1.45]		-0.28	0.07	[-.42 -.15]	
Table 3.9 (Continued)								
Diet diversity								
0 food groups	-1.44	0.27	[-1.96 -.92]	0.127	-0.22	0.23	[-.66 .23]	0.693
1-3 food groups	-1.57	0.09	[-1.74 -1.40]		-0.30	0.07	[-.45 -.16]	
4 plus food groups	-1.88	0.33	[-2.53 -1.22]		0.11	0.30	[-.48 .69]	
Meal frequency (meals per day)								
0-1	-1.13	0.22	[-1.56 -.69]	0.299	-0.42	0.19	[-.80 -.05]	0.818
1-3	-1.72	0.13	[-1.97 -1.47]		-0.29	0.11	[-.51 -.08]	
3	-1.58	0.11	[-1.80 -1.36]		-0.22	0.10	[-.42 -.03]	
Consumption of animal protein								
No	-1.88	0.12	[-2.12 -1.64]	0.906	-0.32	0.11	[-.53 -.11]	0.663
Yes	-1.85	0.13	[-2.11 -1.58]		-0.04	0.12	[-.27 .20]	
SES								
Lower	-1.32	0.13	[-1.58 -1.06]	0.047	-0.34	0.11	[-.56 -.12]	0.303
Middle	-1.69	0.15	[-1.99 -1.38]		-0.23	0.13	[-.49 .03]	
Higher	-1.75	0.13	[-2.00 -1.49]		-0.24	0.11	[-.46 -.02]	
Food security categories								
Mildly food secure or food secure	-1.48	0.11	[-1.70 -1.26]	0.036	-0.32	0.10	[-.51 -.13]	0.286
Moderate or severe food secure	-1.67	0.11	[-1.89 -1.45]		-0.23	0.10	[-.42 -.03]	
Region								
Tigray	-1.87	0.13	[-2.12 -1.61]	0.124	-0.65	0.11	[-.88 -.42]	0.000
SNNP	-1.41	0.10	[-1.61 -1.22]		-0.07	0.09	[-.23 .10]	

Table 3.10: Adjusted means of HAZ and WHZ for 24-59.9 months old children

	HAZ				WHZ			
	Mean	SE	95 % CI	P value	Mean	SE	95 % CI	P value
Water source type								
Improved	-2.15	0.09	[-2.32 -1.98]	0.389	-0.22	0.07	[-.35 -.09]	0.904
non-improved	-2.37	0.10	[-2.57 -2.16]		-0.31	0.08	[-.47 -.16]	
Time to get water and back								
≤ 20 minutes	-2.23	0.09	[-2.41 -2.06]	0.418	-0.24	0.07	[-.37 -.11]	0.433
> 20 minutes	-2.22	0.10	[-2.42 -2.03]		-0.28	0.08	[-.43 -.13]	
Type of toilet used by children								
Improved	-2.18	0.09	[-2.35 -2.00]	0.725	-0.17	0.07	[-.30 -.04]	0.122
non-improved	-2.29	0.10	[-2.50 -2.09]		-0.36	0.08	[-.51 -.21]	
Human feces seen in compound								
No	-2.26	0.09	[-2.42 -2.11]	0.289	-0.25	0.06	[-.37 -.13]	0.789
Yes	-2.07	0.14	[-2.35 -1.19]		-0.30	0.11	[-.51 -.09]	
Animal feces seen in compound								
No	-2.27	0.09	[-2.45 -2.10]	0.015	-0.19	0.07	[-.32 -.06]	0.958
Yes	-2.16	0.14	[-2.36 -1.96]		-0.36	0.08	[-.51 -.21]	
Hygiene index								
0	-2.00	0.12	[-2.24 -1.76]	0.038	-0.08	0.09	[-.27 .10]	0.605
1	-2.13	0.10	[-2.33 -1.93]		-0.19	0.08	[-.34 -.04]	
2	-2.18	0.09	[-2.37 -2.00]		-0.21	0.07	[-.34 -.07]	
3	-2.13	0.08	[-2.28 -1.97]		-0.30	0.06	[-.42 -.18]	
4	-2.33	0.08	[-2.49 -2.16]		-0.31	0.06	[-.42 -.18]	
5	-2.30	0.09	[-2.47 -2.13]		-0.29	0.07	[-.42 -.16]	
6	-2.34	0.09	[-2.52 -2.17]		-0.34	0.09	[-.47 -.21]	
7	-2.44	0.11	[-2.66 -2.22]		-0.34	0.08	[-.50 -.17]	
8	-2.46	0.14	[-2.74 -2.17]		-0.41	0.11	[-.62 -.19]	

Table 3.10: Adjusted means of HAZ and WHZ for 24-59.9 months old children

	HAZ				WHZ			
	Mean	SE	95 % CI	P value	Mean	SE	95 % CI	P value
Breastfeeding								
No	-1.96	0.07	[-2.11 -1.82]	0.000	-0.15	0.05	[-.26 -.04]	0.031
Table 3.10 (Continued)								
Yes	-2.52	0.13	[-2.76 -2.27]		-0.37	0.10	[-.56 -.19]	
Diet diversity								
0 food groups	-4.22	0.97	[-6.12 -2.32]	0.788	-1.03	0.60	[-2.21 -0.15]	0.342
1-3 food groups	-2.23	0.08	[-2.38 -2.08]		-0.28	0.06	[-.39 -.16]	
4 plus food groups	-2.07	0.19	[-2.44 -1.70]		-0.03	0.14	[-.30 .25]	
Meal frequency								
0-2 meals per day	-2.34	0.14	[-2.61 -2.06]	0.339	-0.49	0.11	[-.70 -.28]	0.012
3-4 meals per day	-2.11	0.09	[-2.30 -1.93]		-0.27	0.07	[-.41 -.13]	
5 plus meals per day	-2.34	0.12	[-2.57 -2.11]		-0.15	0.09	[-.33 .02]	
Consumption of animal protein								
No	-2.31	0.09	[-2.47 -2.13]	0.204	-0.30	0.07	[-.43 -.17]	0.333
Yes	-2.11	0.11	[-2.32 -1.90]		-0.19	0.08	[-.35 -.04]	
SES								
Lower	-2.39	0.11	[-2.60 -2.17]	0.111	-0.25	0.08	[-.41 -.08]	0.287
Middle	-2.19	0.11	[-2.41 -1.96]		-0.17	0.08	[-.33 .00]	
Higher	-2.11	0.11	[-2.33 -1.99]		-0.35	0.09	[-.51 -.17]	
Food security categories								
Mildly food secure or food secure	-2.14	0.10	[-2.33 -1.95]	0.610	-0.23	0.07	[-.38 -.08]	0.170
Moderate or severe food secure	-2.32	0.09	[-2.50 -2.14]		-0.29	0.07	[-.42 -.15]	
Region								
Tigray	-2.43	0.10	[-2.64 -2.21]	0.005	-0.52	0.08	[-.69 -.36]	0.015
SNNP	-2.11	0.08	[-2.28 -1.95]		-0.11	0.06	[-.23 .02]	

DISCUSSION

The association of WASH and nutritional outcomes

0 to 5.9 months old infants from households with non-improved water sources and presence of animal feces had higher adjusted mean HAZ, though the differences were not statistically significant from their peers from households with improved water sources and no animal feces (Table 3.8). Differences in water sources facilities might not be critical since the infants were mostly on breast milk (72%). Water quality or limited access may affect child care practices and time resource necessary for complementary food preparation for older children (22) but not for exclusively breastfed 0-5.9 months old. In addition, better water source does not achieve full health benefits if it is not accompanied by improved sanitation and better practices of water storage (23). It is possible that the effect improved water sources were confounded by water storage and handling practices. Among Peruvian children with a home connection of water, those from households without sewage that stored water in small containers were significantly shorter than children from households with sewage that stored water in large containers (23). Poor hygiene predicted lower WHZ ($p= 0.085$) indicating that hygiene might be more important in predicting WHZ in this age group, than other WASH indicators (Table 3.8). A difference of 0.44 in adjusted mean WHZ between infants from the cleanest (hygiene score = 0) and those from the dirtiest household (hygiene score = 8) was reported.

The negative regression coefficients for most of WASH variables in predicting WHZ suggest poorer WASH conditions might be important for 6-11.9 months old. The strong association of recent morbidity with lower WHZ (0.53 Z score decrease with

every increase in number of symptoms in the past two weeks, $p< 0.05$ among 6-11.9 months old) suggests morbidity could be a better indicator of the effects of poor WASH conditions. In addition poor hygiene is marginally associated with lower WHZ ($p =0.080$). However, none of the WASH variables or morbidity is associated with HAZ, suggesting differences in underlying causal mechanisms for wasting and stunting.

Poor personal, household and environmental hygiene was significantly associated with HAZ for children over 2 years old ($p=0.038$), Figure 3.5. A difference of 0.46 Z score is reported for adjusted means of HAZ of children from the dirtiest and cleanest households (Table 3.10). This association was independent of socio-economic status and number of symptoms in the previous two weeks (morbidity). These findings indicate that the association between the hygiene index and HAZ may not be confounded by SES or recent morbidity. Neither household income per head nor maternal education explained the effects of water and sanitation on height in Peru (23). The association between hygiene index and with HAZ was independent of IYCF; hence interventions should target both domains for the greatest benefit in nutrition outcomes.

Presence of animal feces was positively associated with HAZ ($p=0.015$), Figure 3.4. This association was also not confounded by socio economic status. Children from households with animal feces during the time of the survey had 0.11 Z score higher than their peers from households where no animal feces were observed. Based on our personal observations in 28 households in SNNPR during a follow up survey reported as a qualitative report **(Appendix 3.1),** infants in rural Ethiopia are unlikely to crawl frequently in outdoor environment with animal feces. Most of the 28 households had no clearly marked kitchen or household yards that could be easily swept clean. Large

animal corrals were in close proximity to the kitchens, in most households, if not inside the kitchen in a few. During the follow up survey, most of the mothers reported children ≤ 2 years of age do not play or crawl outside or in the kitchen, the areas where animal feces were commonly observed. The observation of large animals in close proximity to the living quarters, which are not clearly spread out, supported the mother's self- report that young children do not play outside.

Animal feces could be an indicator of wealthier households who can afford better diet, hence the positive association observed between presence of animal feces and HAZ for over 24 months old. Sixty percent (n=793) of households who owned 2-5 cows and oxen fed a 24-59 months old index child with animal protein in the past 24 hour at least once compared to 50% (n=793) who had 2-5 cows and oxen ($p<0.001$). Similarly, 65% of households with 1-10 chickens and/ or ducks (n=866) fed a 24-59 months old index child with animal protein in the last 24 hours compared to 55% (n=866) who owned number of chicken and ducks in the same range but did not feed animal protein ($p<0.001$). These high proportions of children fed with animal protein for households who owned animals could explain the positive association between the presence of animal feces with higher HAZ. In addition ingestion of animal protein was associated with high HAZ, though not significantly ($\beta = 0.19$, $p=0.204$).

The generally very small changes in coefficients across the three statistical models suggested WASH association with HAZ and WHZ were independent of IYCF, for less than 12 months old and for 24 to 59 months old children. However, WASH-nutrition outcomes associations were more influenced by IYCF variables in the 12-23.9 months age group. Presence of animal feces was marginally associated with better

outcome (0.53 Z score increase in WHZ), p =0.061, and the association was not independent of IYCF for age group 12-23.9 months. This reiterates that households with animals could afford better diet or even feed older infants with animal protein.

The clear association of poor hygiene with lower HAZ in ≥ 24 months' old children, unlike the younger age groups, could be explained by clustering of negative behaviors. Negative or positive behaviors tend to cluster, both at one point in time and over time (24), such that mothers who engage in negative hygiene behaviors earlier on, may also engage in poor practices in subsequent years and these may extend to other dimensions of caregiving. Therefore, the cumulative negative effect of poor hygiene practices will become more evident after only a certain age, in this case at about 2 years, when the process of stunting is complete.

Association between IYCF indicators and nutritional outcomes

A striking reverse causality is reported for breastfeeding and HAZ for 12-23.9, 6-23.9 months and 24-59.9 months old and less striking for WHZ in 24-59.9 months old. These finding concurs with an earlier findings (25) that children's poor growth and health led to increased breastfeeding and not the reverse. Infants, who appear weak and fall ill often, are likely to be breastfed much longer than healthier infant. Poor mothers are also likely to breastfeed longer than wealthier mothers who might have choices for complementary feeding or bottle feeding.

Our findings are strengthened by the observation that birth size was a strong predictor of poor nutritional outcomes in most of the age groups. Smaller than average and average infants at birth were more likely to be stunted in early childhood compared

to bigger than average. Overall, 38% of all ≤ 5 years old children were classified as average birth size, 31% as smaller than average and 31% as bigger than average.

Exclusive breastfeeding between 0-5.9 months of age is high in these regions (72%) and breastfeeding is much higher for 6-23.9 months old, 94% (Table 3.3). Food security status was associated with HAZ, in 6-23.9 months. Moderately or severely food insecure households were associated with 0.35 less HAZ score compared to their peers from mild or food secure households ($p<0.05$). It is plausible that mothers from food insecure households' breast fed more since they had no options for complementary feeding.

Feeding 4 or more food groups, the cut off for the WHO indicator for minimum diet diversity, was associated with higher HAZ compared to zero food groups ($p = 0.042$) for 6-11.9 months old. This association however should be interpreted with caution since the high coefficient (2.93) suggested an unstable estimate due to low proportion of infants who fell in this category of diet diversity. The high coefficient for 1-3 food groups suggests an association with better HAZ outcomes. Each of the categories (1-3 and 4 or more food groups) were associated with higher HAZ in 6- 23.9 months ($P = 0.030$ and $P= 0.055$ respectively). These results underscore the need of a diverse diet for an age where energy intake, animal protein and micronutrients rich foods need to be timely introduced and incrementally fed during this critical period for growth and development.

With minimal variability in HAZ or WHZ explained by the multivariate modeling with adjusting for confounding variables (highest R squared = 0.28 for WHZ and 0.27 for HAZ) the determinants of poor nutritional status need to be studied in

greater detail, especially risk factors for intra uterine growth restriction. Comparatively, WASH explained more variation in both outcomes than IYCF indicators when each cluster was modeled separately and adjusted for the same covariates (R squared for the WASH model > IYCF model in age groups). This suggests that improving WASH in such a context holds great potential in improving growth outcomes in young children. A recent study reported a significant increase in HAZ among 6-36 months old children (+ 0.33 HAZ, p =0.02) in the WASH intervention group (26). The WASH intervention group was the only one of the four interventions (health, nutrition, WASH and all interventions) targeting a food insecure population in Amhara region, Ethiopia, that had significant increase in HAZ.

CONCLUSION

The hygiene index was a better indicator and the most important predictor of HAZ of the WASH indicators evaluated, especially for over 2 years old children who potentially have been exposed to the cumulative effects of poor environmental hygiene and negative caregiving practices and have reached the maximum linear growth faltering (i.e. stunting). The hygiene index may reflect the household members' knowledge and capacity to keep their environment clean. As a more comprehensive measure that reflects knowledge and behavior, the hygiene index can be a good indicator of mother's overall capacity and caregiving practices that may affect other dimensions like IYCF. Since this index included many different attributes likely to co-vary in the same direction, it is a better measure than spot check surveys for presence of feces in the compound. However, the positive association of the presence of animal feces and better growth outcomes need to be investigated further. Repeated observations

for sanitation indicators such as presence of feces are recommended. Assessment of WASH need to be complemented with in depth observation since certain WASH related practices, like hand washing, fecal disposal and cleaning of compound vary and cannot be representatively captured in one time point.

Improvements in WASH are likely to have additive growth benefits independent of IYCF for ≤12 and ≥ 24 months' old children in rural Ethiopia context. Among the 12 to 23.9 months old, interventions targeting WASH might have a synergistic effect with IYCF as suggested by the non-independence of the two clusters of indicators in this cross sectional data analyses. It is therefore imperative that more effort on interventions and research to generate more evidence on what works be devoted to WASH in the context of infant feeding as a focal determinant of malnutrition. A more holistic approach to WASH is critical. Household environment hygiene education should complement Community Led Total Sanitation (CLTS) initiatives and water supply and quality improvement interventions. Hygiene education should emphasize household environmental hygiene in addition to hand washing with soap

Acknowledgements

We thank Purnima Menon for the opportunity to collaborate on the Alive and Thrive baseline data analyses. We also thank Purnima, Disha Ali, Rahul Rawat and Michael Tedla at IFPRI, for the support and advice during the data analysis. This research was funded by Borlaug Leadership in Agriculture Enhancement Program (LEAP), University of California, Davis, USA.

APPENDIX

Appendix 3.1: Qualitative report from a field visit to SNNPR - Household Observation on WASH

We visited 28 households (HH) in two *Kebeles* (Debo and Gololcha) and conducted 15-20 minutes household spot checks and informal interviews with the mothers.

Kebele 1 – Debo

A total of 13 households were visited in several *Gottes*. The residents were mostly Subsistence farmers (cereals) and cattle rearing. They also have coffee farms elsewhere.

Water sources

A protected community borehole and government provided tap water traded by one household at a small shopping center was the main water source. The furthest house to visit was not more than an hour from the water source. Most of the 13 HH had a narrow mouth 20 liter plastic container for water storage, except two which had a wide mouth. Water guard use was reported in all the households visited in Debo except for one who had run out of water guard. They said they are boiling drinking water before they receive the next donation of water guard.

Water service items (commonly plastic jugs) were visibly dirty even though most households had narrow mouth containers and scooping of water was not likely.

Sanitation

Majority of the HH had pit latrine toilet credited to Community Led Total Sanitation (CLTS) promoted by various collaborators; however, the latrines were unappealing and dangerous for children's use. Some latrines were constructed with two squat holes; a small one for children and a large one for adults. The laying of logs or wood for the floor material was in most cases not well done and left openings that could trap a child's foot. Commonly the latrines were makeshift structures with no roof (in all households in Debo) and at times no walls (a third of the households). Walls for the latrines were made of bamboo or nylon bags or "*false banana"* leaves or the "*false banana"* plants. No human feces were observed around the household area.

The households had no clear kitchen yards and animal feces (cattle, donkey and dogs) were common in the immediate surroundings of the HH. The area around the houses was commonly very shaded and wet and the environment was generally very dirty. It was very hard to keep the household environment clean since there was no clearly defined yard that could be easily swept. The area around the HH had either grass or it was the cattle feeding area and had a lot of animal feces. One of the households reported to leave the feces in the open on purpose to allow them to dry for collection later on and disposal onto the farm as manure.

The field staff that guided our visits and assisted with interpreting had this to say when she noticed that we were very keen on the HH environment and animal feces *"The false banana (which is the staple for most people in this area) "live" in a better place than humans".*

Nappy wash was done on the grass outside the main house and waste water thrown into the field and/or garden in some households. In a third of the HH mothers took laundry and nappies to the river.

Indoor environment

Two thirds of the HH had floors made of the bamboo mat/carpet and the rest were either earthen or made of wood. Bamboo mat floors were very hard to sweep clean since soil was easily trapped in between the interwoven bamboo bark One living house had a cemented floor and one had an earthen floor covered with a plastic carpet (easy to clean). Wooden floors were also unkempt, soiled and wet at times and infants were found to play and crawl on these during the observation of the households.

Most of the kitchens were small, with dirty and wet earthen floors and had animal feces (cow dung). The kitchens were very poorly lit and had poor indoor air quality (no ventilation). All the mothers reported that the child did not play or get to the kitchen. A reason given for keeping away children from the kitchen was to protect them from the fire. When asked where the child usually plays, the mothers said outside or in the living house. The areas surrounding the living house and the kitchen had animal feces in all the households and within the immediate reach of a crawling infant.

Food storage areas and tables were frequently soiled and dusty. Many of the households prepare fresh food for the baby (no storage of food).

Generally animal feces (cattle: cows, sheep and goats), donkeys and horses were observed in all households in very close proximity to the living house and kitchen area. Most people in this area have managed to separate the animal corral from the kitchen

within the past one decade as advocated by the government and various partners, however, the proximity of the animal corral or the animal feeding areas leaves the household environment heavily contaminated. Community led total sanitation (CLTS) was successful in promoting latrines construction (despite their poor state) but animal feces disposal was inadequate. Chicken feces were only observed in two households in this *Kebele*. Poultry farming was not common in the area.

Most of the households (90 %) owned a coloured TV and a radio, yet the latrines were so unappealing to use, in fact dangerous to use for children.

Kebele 2- Gololcha

We visited fifteen households in Gololcha. Most of the residents were coffee farmers. About half of the households owned cattle. A third of the households (5/15) with clearly defined, easy to sweep yards. The households' environment was much cleaner compared to households in Debo. The environment had little or no animal droppings less than half of the households.

Latrines were similar to those in Debo and about half had no walls. Two households shared latrines with their neighbors or grandparents. Most of the HH store water in narrow mouth containers but the water service items (plastic jugs) were visibly dirty.

The interior of living houses had dirty floors mostly made of the bamboo carpet (soiled and with wet spots at times) or earthen (wet in most cases and seemed hard to dry because of the shaded area surrounding most houses). Child play areas were less contaminated with animal feces compared to Debo.

Households in Gololcha were far away from the nearest shopping center or tarmacked road (about 5 km for the nearest). They were much poorer in terms of electronic assets and animals compared to the HH in Debo which were close to the tarmacked road.

Three households had animal corral (calves, goats and sheep) as a partition in the kitchen which was the tradition a decade ago to avoid theft. Many of the households have separated the animals from the kitchen and though this was one of the success stories (in addition to the CLTS promotion of latrines construction and proper human feces disposal), there was still a lot to be done to separate the animals from the living quarters and the child's environment. Poultry was observed in one household in this Gololcha.

All households treated water with Waterguard except for one which reported they did not need to treat drinking water since it was tap water.

Mothers in both *Kebeles* have a good knowledge of hand washing before or after key events (after toilet use, before feeding the child or preparing food). However, a hand washing station next to the toilet was only observed in three out of the 28 households and soap was only found in two of the stations.

Nappy wash was commonly done in the River at Gololcha. Nappies were commonly used in the night when the baby was sleeping, otherwise the children were left to play around with no nappy or pants during the day and once they defecated the mother scooped the feces and disposes them into the latrine. Mothers reported

supervising older children to use the latrine whereas older infants were helped to squat on the mothers feet with "*false banana"* leaves as the "potty" underneath.

Conclusion

Contamination of household environments with animal feces was prevalent despite the success with human feces disposal. Water handling (re-contamination) might present a threat to the child's health since most of the water service items were clearly visibly dirty even though knowledge on water treatment using Water Guard was high. Lack of hand washing stations and soap at strategic places (near latrines) raised questions on whether the self-reported knowledge on key events for hand washing really translated to the practice (hand washing with soap). Latrine use by young children was doubtful since most of the latrines were so unappealing and dangerous for the children. Even though mothers said they supervised the young children to use the toilet, several mothers reported time was a constraint to keeping the indoor and outdoor environment clean. They were either busy in the farm or at times trading in the nearby shopping center.

We sought to know from one mother, why her household indoor (living room floor) and outdoor environment was in such a dirty state and she said:

"I know I should clean my house and surrounding area, but I do not have much time. If I stay at home doing the cleaning, my children cannot eat the food from the false banana only, they need something else in addition and so I have to work on the false banana "product" and go trade to be able to buy something else for my children"

Very few mothers practiced finger feeding, another success story credited to the promotion of spoon feeding by the Community Health Extension Workers.

REFERENCES

1. Black RE, Allen LH, Bhutta ZA, Caulfield LE, Onis M, Ezzati M, Mathers C, Rivera J (2008). Maternal and child undernutrition: global and regional exposures and health consequences. *Lancet*; 371: 243-260.
2. Victora CG, Adair L, Fall C, Hallal PC, Martorell R, Richter L, Sachdev HS (2008). Maternal and child undernutrition: consequences for adult health and human capital. *Lancet;* 371(9609):340-57.
3. Penny ME, Creed-Kanashiro HM, Robert RC *et al*. (2005). Effectiveness of an educational intervention delivered through the health services to improve nutrition in young children: a cluster-randomised controlled trial. *Lancet*; 365, 1863–1872.
4. Victora CG, de Onis M, Hallal PC, Blössner M, Shrimpton R (2010). Worldwide timing of growth faltering: revisiting implications for interventions. *Pediatrics*; 125(3):e473-480
5. Raikumar AS, Gaukler C, Tilahun J (2012). Combating malnutrition in Ethiopia: an evidence-based approach for sustained results. *African human development series,* World Bank.
6. Daniels DL, Cousens SN, Makoae LN & Feachem RG (1991). A study of the association between improved sanitation facilities and children's height in Lesotho. *Eur. J. Clin. Nutr.* 45,23–32.
7. Magnani RJ, Mock NB, Bertrand WE & Clay DC (1993). Breastfeeding, water and sanitation, and childhood malnutrition in the Philippines. *J. Biosoc. Sci.* 25, 195–211.

8. Ricci JA & Becker S (1996). Risk factors for wasting and stunting among children in Metro Cebu, Philippines. *Am. J. Clin. Nutr.* 63, 966–975.
9. Sedgh G, Herrera MG, Nestel P, El Amin A Fawzi WW (2000). Dietary vitamin A intake and nondietary factors are associated with reversal of stunting in children. *J. Nutr.* 130, 2520–2526.
10. Esrey SA (1996). Water, Waste, and Well-Being: A Multicountry Study. *Am J Epidemiol;* 143:608-23
11. Esrey SA, Habicht JP, Casella G (1992). The complementary effect of latrines and increased water usage on the growth of infants in rural Lesotho. *Am. J. Epidemiol.* 135, 659–666.
12. Merchant AT, Jones C, Kiure A, Kupka R, Fitzmaurice G, Herrera MG, Fawzi WW (2003). Water and sanitation associated with improved child growth. *European Journal of Clinical Nutrition.* 57, 1562–1568.
13. Humphrey JH (2009).Child undernutrition, tropical enteropathy, toilets, and hand washing. *Lancet;* 374: 1032--1035
14. http://measuredhs.com/what-we-do/survey/survey-display-359.cfm
15. International Food Policy Research Institute (2010). Alive and Thrive Ethiopia, Baseline Survey Reports: Methods Sections, IFPRI.
16. Moret L, Mesbah M, Chwalow J, Lellouch J (1993). Internal validation of a measurement scale: relation between principal component analysis, Cronbach's alpha coefficient and intra-class correlation coefficient. *Rev Epidemiol Sante Publique*; 41:179–86.
17. Bland JM, Altman DG (1997). Statistics notes: Cronbach alpha. *BMJ*; 314:572

18. World Health Organization (2008). Indicators for assessing infant and young child feeding practices: conclusions of a consensus meeting held 6–8 November 2007 in Washington D.C., USA.
19. Ruel MT, Menon P (2002). Child feeding practices are associated with child nutritional status in Latin America: innovative uses of the demographic and health surveys. *J. Nutr.* 132: 1180–1187.
20. Shrimpton R, Victora CG, De Onis M, *et al.* (2001). Worldwide timing of growth faltering: implications for nutritional interventions. *Pediatrics,* 107: E75.
21. Central Statistical Agency [Ethiopia] and ICF International (2012) Ethiopia Demographic and Health Survey 2011. Addis Ababa, Ethiopia and Calverton, Maryland, USA: Central Statistical Agency and ICF International.
22. Burger SE, Esrey SA (1995). Water and sanitation: health and nutrition benefits to children. In: Pinstrup-Andersen P, Pelletier D, Aldermann H, eds. Child growth and nutrition in developing countries: priorities for action. Ithaca, NY: Cornell University Press: 153-75.
23. Checkley W, Gilman RH, Black RE, Epstein LD, Cabrera L, Sterling CR, Moulton LH (2004). Effect of water and sanitation on childhood health in a poor Peruvian peri-urban community. *Lancet;* 363: 112–18.
24. Arimond M, Ruel MT (2001). Assessing care: progress towards the measurement of selected childcare and feeding practices, and implications for program. Food Consumption and Nutrition Division, Discussion Paper # 119. International Food Policy Research Institute, Washington, DC.

25. Marquis GS, Habicht J-P, Lanata CF, Black RE, Rasmussen KM (1997). Association of breastfeeding and stunting in Peruvian todders: An example of reverse causality. *International Journal of Epidemiology;* 26: 349–356.
26. Fenn B, Bulti AT, Nduna T, Duffield A, Watson F (2012). An evaluation of an operations research project to reduce childhood stunting in a food-insecure area in Ethiopia. *Public Health Nutrition.* doi:10.1017/S1368980012001115.

CHAPTER 4

AFLATOXIN EXPOSURE AMONG MOTHERS IS ASSOCIATED WITH SEVERE STUNTING IN 6-59 MONTH OLD CHILDREN IN ZIMBABWE

ABSTRACT

Aflatoxins are common staple food contaminants produced by *Aspergillus flavus* and *Aspergillus parasiticus*. Dietary exposure to aflatoxins was associated with growth impairment in children in several West African countries. However, data is lacking on the extent and geographic distribution of aflatoxin exposure in many countries, including Zimbabwe. We conducted a cross-sectional study to assess the extent of recent aflatoxin exposure in 287 women of child bearing age from a representative survey of all ten provinces in Zimbabwe. 17% (n=287) of the women had detectable levels (≥ 0.15 ng/ml) of urinary aflatoxin M1 (AFM1). Urinary AFM1 from 199 mothers was used as a proxy for aflatoxin exposure among their children. Detectable aflatoxin exposure was associated with severe stunting in 6- 59 months old children (odds ratio =3.30, 95% CI: 0.98 – 11.13, p=0.055). A dose response relationship between exposure tertiles and odds of severe stunting was observed (P=0.035). The biological mechanisms underlying the association remain unclear and require further studies. Our findings point to a significant public health problem in rural Zimbabwe. Low cost and effective intervention measures to prevent aflatoxin exposure might be critical in the prevention of childhood stunting.

INTRODUCTION

Maize and groundnuts are important components of human diets in rural Zimbabwe and excellent substrates for fungi that produce secondary metabolites known as mycotoxins, which are harmful to animal and human health. Of the mycotoxins, aflatoxin is of particular concern in sub Saharan Africa because it is a common food contaminant (1) and several outbreaks of acute aflatoxicosis have been reported (2). It is a hepatotoxic, carcinogenic, immunosuppressive and anti-nutritional contaminant of many staple food commodities (1).

Gong et al. have reported that exposure to aflatoxin was associated with growth failure in young children in Benin and Togo (3, 4, 5). The carcinogenic properties of aflatoxin are relatively well documented in humans (6). In contrast, the effects on growth, immune status and susceptibility to infectious disease, though well recognized in farm animals (7), are less explored in human populations. Chronic exposure to aflatoxin interferes with protein metabolism and multiple micronutrients that are critical to health in farm and laboratory animals (1). These anti-nutritional effects have been poorly studied in humans.

The underlying mechanisms associated with growth failure may also include compromised immunity and increased susceptibility to infectious diseases (8). Aflatoxins might increase intestinal permeability by their toxic effects on the tight junction proteins of the small intestine mucosa lining (9). The loss of the gut barrier function predisposes young children to environmental immunogenic macromolecules (e.g., other microbial toxins or bacteria) which can enter the circulation through a highly

permeable gut caused by chronic inflammation of the mucosal lining (10). These harmful toxins can in turn cause chronic immune stimulation and diversion of energy from growth. Loss of absorptive capacity from villous atrophy could also lead to decreased absorption capacity for various nutrients, contributing to malnutrition (10). In Gambian infants, greater than 43% of long-term growth faltering for the first 15 months could be explained by the presence of small intestinal mucosal enteropathy (11). Aflatoxin exposure may contribute to this condition (12).

Contamination of food staples with aflatoxin occurs as a result of fungal action (*Aspergillus flavus and Aspergillus parasiticus)* pre- and post- harvest and during storage. Accumulation of aflatoxin is exacerbated when crops are subjected to drought stress and when foods are stored under sub-optimal conditions, i.e. temperatures between 24 and 35 ^{o}C and moisture content exceeding 7%. These conditions are frequently encountered in Zimbabwe, yet awareness of the problem is low, no surveillance measures are in place, and little is being done to ensure food safety. We aimed to find out whether aflatoxin exposure was prevalent in Zimbabwe, and whether it was associated with growth faltering in young children.

Study Population and Sampling Frame

Urinary level of Aflatoxin M1 was assayed in 287 archived urine samples from women 15-49 y old) in households with <5 y old children whose anthropometry data were collected in a national micronutrients survey in October-December 2008 in Zimbabwe. The urine samples were initially collected to test for a parasite (*Schistosoma Haematobium)* as part of a national micronutrient survey, and the remaining urine was

stored at -80 C in carefully monitored freezers. Multistage sampling was used to select enumeration areas (EAs), households and respondents for the national survey. Stratification was done at two levels to provide reliable estimates for each province and socioeconomic/ land use sectors.

METHODS

Aflatoxin M1 concentration in urine was determined using direct enzyme-linked immunosorbent assay with a high affinity (mean binding of 98%), mean recovery rate of 96% and detection limit of 0.15 ng/ml, for aflatoxin M1 (Helica Biosystems, INC). The development of simple, sensitive and specific enzyme-linked immunosorbent assays has enabled assessment of aflatoxin and its metabolites in body fluids at population levels compared to more expensive methods, such as high performance chromatography (HPLC), which require purification of the biomarker of interest (13, 14, 15).

AFM1 in urine provides a good indicator of AFB1 consumption over the previous 3 days (16) and about 2% of ingested AFB1 appears as AFM1 in urine (17). We chose this approach rather than sampling food because a representative sample is nearly impossible to obtain due to the non-uniform distribution of the toxin. A good correlation (correlation coefficient for the linear regression equation =0.66) has been reported for total dietary aflatoxin B and urinary AFM1 in a three day study (17). In our study women with detectable levels of urinary AFM1 (i.e. $\geq$ 0.15 ng/ml) were classified as exposed. Undetectable levels were considered as unexposed.

Data Analysis

Multivariate logistic regression was carried out with stunting (height for age Z scores, HAZ, < -2) or severe stunting (HAZ < -3) as the outcome of interest and urinary aflatoxin M1 as the independent variable. Aflatoxin M1 was included in the regression model as a dichotomous variable (exposed and non-exposed categories). To test a dose response relationship the independent variable was included as tertiles of the exposed and these were compared to the non-exposed category as the reference. Of all the 287 archived urine samples of women of child bearing age, 199 samples were linked to a child <5 years of age. Thus multivariate analysis was carried out with an overall sample size of 199 mother-child dyads. HAZ scores were created using WHO growth standards (18). Covariates available for multivariate modeling included mother's marital status, education, body mass index, age, level of education, parity, birth size, age, sex of the child. All data analyses were carried out using STATA statistical software, Version 10 (College Station, Texas, USA).

RESULTS

Of the 287 women assessed, 17% (50/287) had detectable urinary Aflatoxin M1 levels [mean (SD) value for the exposed: 0. 39 (0.60) ng/ml]. The range among the exposed (0.15- 3.74 ng/ml) was similar to the range seen in a comprehensive survey of a Chinese population at risk of liver cancer, 0.15- 4 ng/ml, (19). The exposed mothers did not differ significantly from the unexposed in any maternal characteristics **(Table 4.1).** 64% of the mothers had attended secondary school. The exposed households had higher proportions of stunted and severely stunted children, though the proportions were not significantly different from the unexposed households. Mean HAZ score was non-

significantly lower (-1.64 vs -1.39, p=0.257) among exposed children. The highest proportion of stunted and severely stunted children occurred in the age group 12-23.9 months (38 and 16% respectively) **Table 4.2**.

Table 4.1: Maternal and child characteristics by aflatoxin exposure (n =199)

Characteristics	Total (n=199)	Exposed (n=35)	Unexposed (n= 164)	P value
Maternal characteristics				
Mean BMI (SD)	16.16±1.96	16.14±1.37	16.16±2.07	0.950
Age in years, mean (SD)	27.51±7.32	27.37±7.95	27.54±7.20	0.904
Married, n (%)	140 (70.40)	21 (60.00)	119 (72.60)	0.140
Highest level of education, n (%)				
Primary	59 (31.40)	10 (30.30)	49 (31.61)	
Secondary	121 (64.40)	22 (66.70)	99 (63.90)	
Higher	2 (1.10)	0(0.00)	2 (1.30)	0.924
Years completed at highest level of school, mean (SD)	4.62±2.26	4.24±2.55	4.65±2.20	0.591
Parity (mean±SD)	3.09±1.91	3.15±1.94	3.08±1.91	0.847
Child characteristics				
Age in months (mean±SD)	28.94±14.51	27.40±14.93	29.27± 14.44	0.489
Birth weight, g (mean±SD)	3100±524	3091±491	3102±533	0.932
Birth size (mean±SD)	2.93±1.39	2.71±0.87	2.98±1.47	0.292
Birth size, median (Range)	3(1-9)	3(1-5)	3(1-9)	
WHZ score (mean±SD)	-0.06±1.07	-0.07±0.90	-0.05±1.10	0.930
HAZ score (mean±SD)	-1.43±1.19	-1.64±1.30	-1.39±1.17	0.257
Not stunted, n (%)	137 (68.80)	21 (60.00)	116 (70.70)	
Stunted, n (%)	62 (31.20)	14 (40.00)	48 (29.30)	0.213
Severely stunted, n (%)	19 (9.60)	6 (17.10)	13 (7.90)	0.092

Table 4.2: Percent stunted and severely stunted by age category (n=199)

Age Category	Mean HAZ (SD)	N	Percent stunted	Percent severely stunted
6-11.9	-0.87 (1.13)	26	19	0
12-23.9	-1.52 (1.34)	58	38	16
24-35.9	-1.73 (1.01)	55	35	11
36-47.9	-1.33 (1.02)	31	26	7
48-60	-1.31 (1.28)	29	28	7

In multivariate analyses, aflatoxin exposure among mothers was marginally associated with severe stunting in their 6 to 59 months old children (adjusted odds ratio 3.30, p=0.055), **Table 4.3**. A significant dose-response relationship for the odds of severe stunting with increase in exposure level is demonstrated **(Figure 4.1),** p=0.035. Aflatoxin exposure varied by region (p<0.001) **(Figure 4.2)**. Mashonaland west and Manicaland provinces (North West and South East of Harare) had the highest percentage of women with detectable levels of AFM1. The lowest percentage of exposed was in Harare (2%). Bulawayo city and province, the other major urban area of the country, had a higher percentage of exposed women compared to Harare (17%).

Table 4.3: The association between aflatoxin exposure and stunting or severe stunting in 6-59 months old

Outcome	Unadjusted Odd Ratio (95 % CI), n= 199	p value	Adjusted Odds Ratio (95 % CI), n= 184	p value
Stunting	1.61 (0.76- 3.42)	0.216	1.41 (0.61 - 3.25)	0.427
Severe stunting	2.40 (0.84 – 6.84)	0.100	3.30 (0.98 – 11.13)*	0.055

Adjusted odds ratio were adjusted for birth size, sex of the child, age of the child, mother's age, level of education, marital status, BMI and parity. *n=178 for adjusted model with severe stunting as the outcome variable

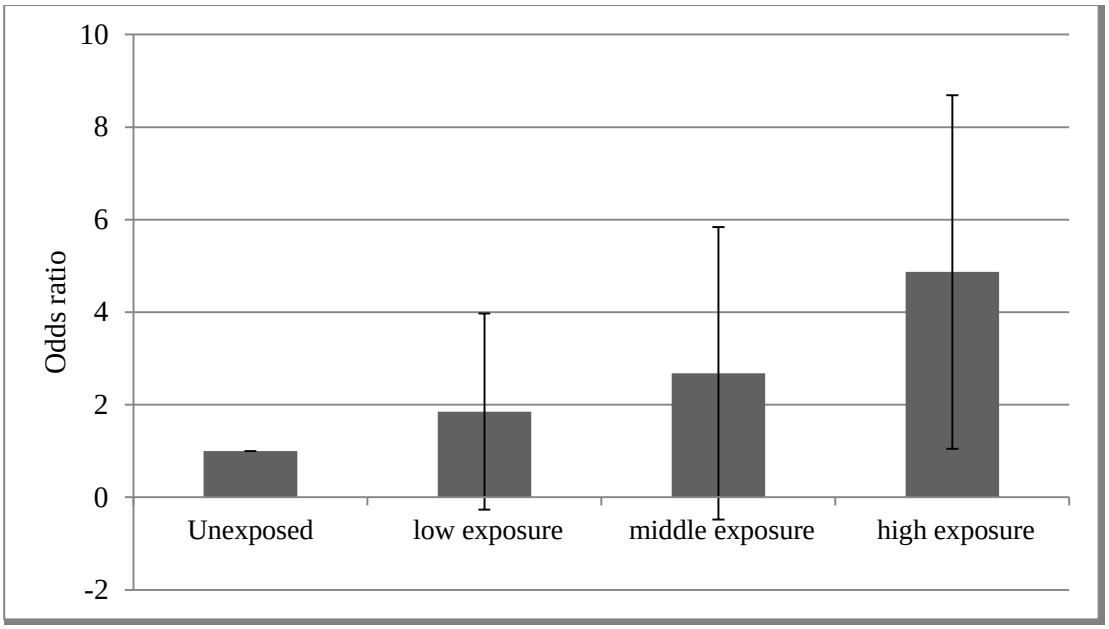

Figure 4.1: Odds of severe stunting with exposure category (6-59 months old)

Odds ratio are adjusted for birth size, sex of the child, age of the child, caregiver's age, and level of education, marital status, BMI and parity, P value for the trend = 0.035. n= 12 for low and middle exposure category; n=11 for high exposure and n= 164 for unexposed. The aflatoxin concentrations in the tertiles were: 0.15- 0.18 ng/ml in the low tertile, 0.19-0.34 ng/ml in the middle tertile and 0.35-3.70 ng/ml in the high tertile/ exposure.

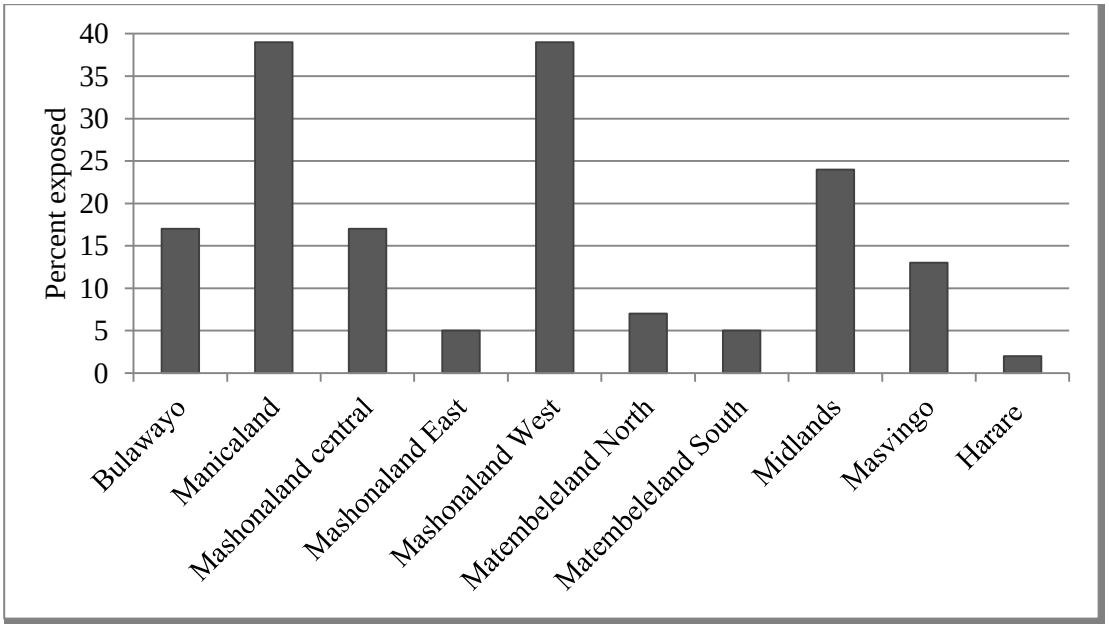

Figure 4.2: Percent of exposed women by province

Bulawayo; n=36, Manicaland; n=28, Mashonaland Central; n=30, Mashonaland East; n=22, Mashonaland West; n=23, Matembeleland North n=28, Matembeleland South; n=21, Midlands; n=34, Masvingo; n=16, Harare; n=49.

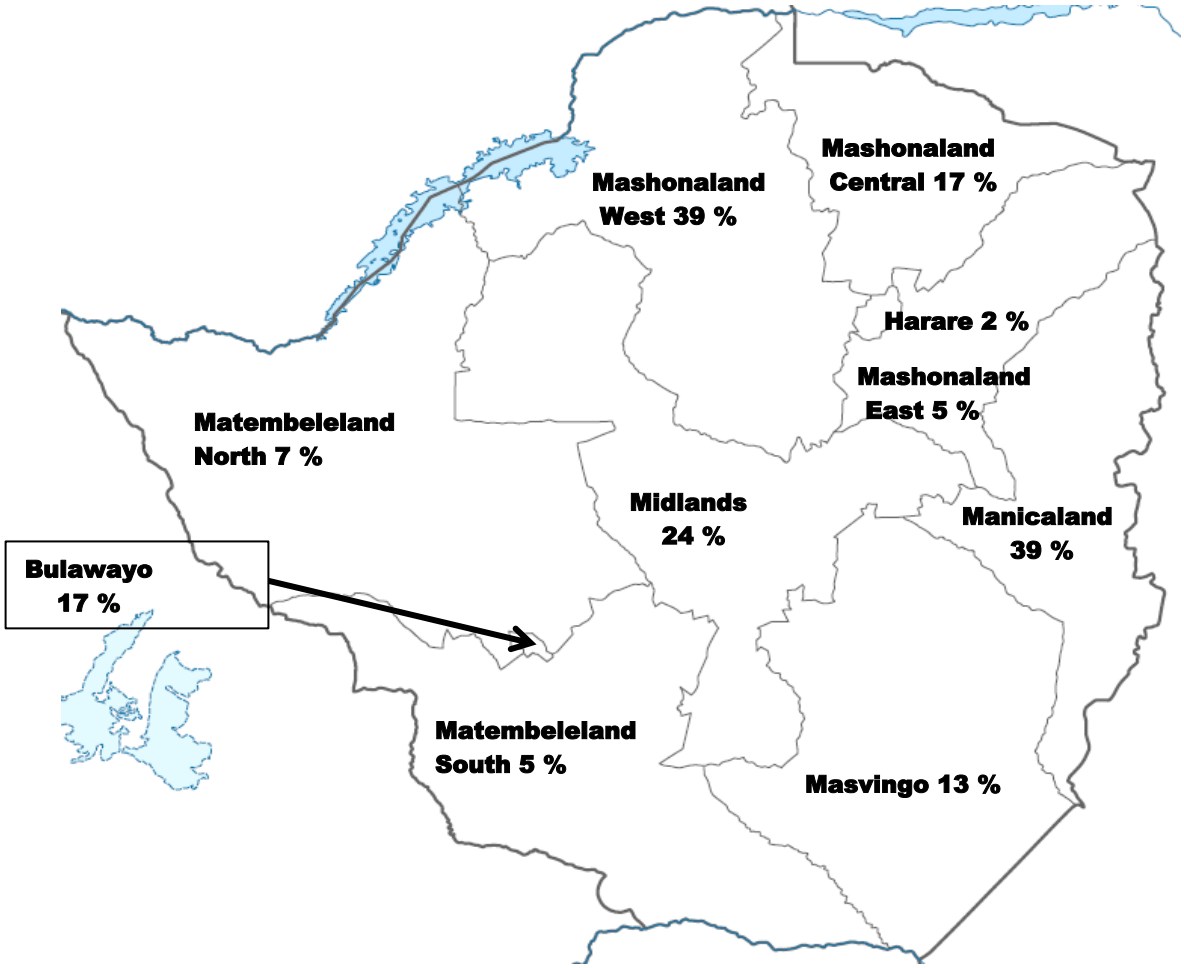

Figure 4.3: Aflatoxin B1 exposure distribution by region for all women

DISCUSSION

These data signal a significant aflatoxin exposure in Zimbabwean households, indicating that 17% of the sample of adult women consumed AFB1 in the 3-d period prior to the survey. As far as we know, these are unique data within Zimbabwe, and they provide an impetus for an urgent multi-sectoral approach to minimizing the health burden from aflatoxin. While acute outbreaks of aflatoxicosis make the headlines, as was the case in 2004 and 2005 in Kenya, aflatoxin control should not only focus on acute exposures. Chronic exposure is certainly much more prevalent and may be a much larger contributor to childhood stunting and other disease conditions. Stunting is a process that begins in utero and is essentially complete by 2 years of age (20). Thus the association with severe stunting, if truly causal, suggests that the children of the exposed mothers could have been exposed *in utero* or in early childhood during complementary feeding.

Chronic exposure to aflatoxin in food has a synergistic effect with hepatitis B virus (HBV) in causing liver cancer (21) and has far reaching health implication particularly for the 20% of people in developing countries with 20% HBV (1). Since aflatoxin decreases the body's immune defenses a significant reduction in survival time has been hypothesized for people living with HIV (1).

This study has several strengths and limitations. The relatively large sample size (n=287) is drawn from all the ten provinces in Zimbabwe. Our sample sizes for each province were based on availability of anthropometry data for under 5 y old children of the same households. The number of women from each province may not be representative of the population. Although aflatoxin exposure assessment was a

secondary analysis based of the initial national survey, our results show high exposure rates and an association with severe stunting in 6-59 months old.

We adjusted for many maternal and child characteristics, however a wealth index could not be constructed due to the limitations of the survey that provided our sample. The multivariate regression models were adjusted for maternal education as a proxy for socio-economic status. Previous studies share this limitation (22). In the context of rural African poverty, income or wealth is difficult to measure reliably and may vary too little to be a large predictor of child stunting. Other studies that adjusted for economic variables did not find that this explained the association between aflatoxin exposure and growth association (3, 4). A dose response relationship between the tertiles of exposure level and odds of severe stunting add to the strength of the associations reported.

The cross-sectional design does not elucidate whether the aflatoxin exposure occurred prior to the process of linear growth retardation. A strong negative correlation between aflatoxin exposure and height increase over an 8-month period in a longitudinal study of two hundred West African children 16-37 months of age strongly indicates aflatoxin exposure precede growth faltering (5). Numerous animal experiments have demonstrated association between aflatoxin exposure and growth impairment (7), although the underlying mechanisms remain unclear.

The urinary biomarker that we used was simple and low-cost, but reflects only recent intake. Given the non-uniform distribution of aflatoxin in food, aflatoxin albumin adduct measured in blood has an advantage of showing cumulative exposure

over 2-3 months as opposed to the urinary measure we analyzed. Assessment of AFM1 in urine collected from the mothers was used as a proxy for aflatoxin exposure among their young children. Diet variation among household members in poor rural households is minimal due to income constraints. Most young children are fed from the family pot and therefore the use of maternal urinary AFM1 as proxy for the child's dietary exposure was plausible. To the extent that the use of a short-term biomarker and maternal specimens led to misclassification of exposure, this would have attenuated the association that we observed. Thus the relationship we report may be an underestimation of the true association.

Finally, geospatial and seasonal distribution of these contaminants of staples should be studied further in Sub-Saharan Africa to provide data and evidence for targeting interventions. A more rigorous sampling frame across agro-ecological zones and seasons is recommended to achieve a more representative measure of the health burden from aflatoxin exposure. Seasonal and geographic variations have been reported in West Africa (5). Urine sampling for this study was during the months of September to November 2008, the onset of the rainy season in Zimbabwe, and likely to coincide with a season of high aflatoxin levels from accumulation in inadequately stored grains harvested in May and June. However, maize harvests across the country were very low due to floods in December 2007 and January 2008, followed by dry spells in several provinces. In addition, low purchasing power and lack of access to farm inputs due to the fast economic decline and hyperinflation facing the country in the year 2007-2008 could have contributed to the low harvest. Small scale holder farmers might have exhausted their food stores much earlier and were likely to rely on imported food or

food aid from the humanitarian organizations like World Food Program (WFP). It is therefore difficult to know how our results compare to the current condition.

On a national policy level, political will and capacity development for routine food safety monitoring and enforcement of regulations is critical for such a potent food contaminant that could have far reaching national and regional health and economic implications. A collaborative approach to mitigating the known and unknown health burden from aflatoxin is needed in Zimbabwe. A strong food safety monitoring and database is critical at a time when policies geared towards food redistribution to hard hit areas within a country, and in Africa at large, are indispensable. The quick inexpensive ELISA method that we used here could be adapted for routine monitoring of aflatoxin exposure by analyzing urine samples collected for example during pregnancy care.

Low-technology intervention measures can substantially reduce exposure to aflatoxins (23). These approaches include training small scale farmers on hand sorting techniques for moldy or damaged groundnuts kernels. Such a simple measure could protect household members and improve the safety of home processed pea nut paste for small scale traders. Other low cost and effective intervention measures include training farmers on how to judge adequate sun drying of ground nuts and maize, sun drying on natural natural-fibre mats, proper storage of grains in natural-fibre bags and on wooden pallets can prevent accumulation of moisture in grains (23). Use of insecticides on storage areas (floors) and wooden pallets can prevent insects which produce moisture by their metabolic activity and spread mold spores. Adequate measures to completely destroy contaminated crop should be established to remove such from the food chain.

In conclusion, dietary aflatoxin exposure is a significant public health concern in rural Zimbabwe and exposure among mothers is associated with severe stunting in their children. These findings suggest that chronic exposure might be common given the prevalent dietary patterns, post-harvest practices and climatic conditions. Assessment of household exposure to Aflatoxin can be done inexpensively from urinary collections at health clinics for surveillance.

Acknowledgements

We thank Zvitambo Research project for supporting this research. This research was supported by funds from First Presbyterian Church, Ithaca, USA.

REFERENCES

1. Williams JH, Phillips TD, Jolly PE, Stiles JK, Jolly CM, Aggarwal D (2004). Human aflatoxicosis in developing countries: A review of toxicology, exposure, potential health consequences, and interventions. *Am J Clin Nutr*; 80:1106–22.
2. Shephard GS (2008). Risk assessment of aflatoxins in food in Africa. *Food Additives and Contaminants Part a-Chemistry Analysis Control Exposure & Risk Assessment* 25:1246-56.
3. Gong YY, Cardwell K, Hounsa A, Turner PC, Hall AJ, Wild CP (2002). Dietary aflatoxin exposure and impaired growth in young children from Benin and Togo: cross sectional study. *BMJ*; 325:20 –1.
4. Gong YY, Egal S, Hounsa A *et al.* (2003). Determinants of aflatoxin exposure in young children from Benin and Togo, West Africa: the critical role of weaning. *Int J Epidemiol*;32: 556–62.
5. Gong YY, Hounsa A, Egal S, Turner PC, Sutcliffe AE, Hall AJ, Cardwell K, Wild CP (2004). Postweaning exposure to aflatoxin results in impaired child growth: A longitudinal study in Benin, West Africa. *Environ Health Perspect*; 112:1334–8.
6. Gorelick NJ, Bruce RD, Hoseyni MS. Human risk assessment based on animal data: inconsistencies and alternatives. In: Eaton D, Groopman JD, eds. (1993).The toxicology of aflatoxins: human health, veterinary, and agricultural significance. London: Academic Press, 1993:508 –11.
7. Khlangwiset P, Shephard GS, Wu F (2011). Aflatoxins and growth impairment: a review. *Critical Reviews in Toxiology*; 41(9): 740-755.

8. Wild PC (2007). Aflatoxin exposure in developing countries: The critical interface of agriculture and health, *Food and nutrition bulletin*; 28 (2) S372-S380.
9. McLaughlin J, Padfield PJ, Burt JP, O'Neill CA (2004). Ochratoxin A increases permeability through tight junctions by removal of specific claudin isoforms. *Am J Physiol Cell Physiol*; 287:C1412–7.
10. Campbell DI, Elia M, Lunn PG (2003). Growth faltering in rural Gambian infants is associated with impaired small intestinal barrier function, leading to endotoxemia and systemic inflammation. *J Nutr*; 133:1332–8.
11. Lunn PG (2000). The impact of infection and nutrition on gut function and growth in childhood. *Proc Nutr Soc*; 59:147–54.
12. Smith LE, Stoltzfus RJ, Prendergast A (2012). Food chain mycotoxin exposure, gut health, and impaired growth: a conceptual framework. *Adv. Nutr***.** 3: 1–6.
13. Chu, F. S (1984). Immunoassays for analysis of mycotoxins. *J. Food Prot.,* 47: 562-569.
14. Chu, F. S. Recent studies on immunochemical analysis of mycotoxins. In: P. Steyn and R. Vleggaar, eds. (1986). Mycotoxins and Phycotoxins, pp. 277-292. Amsterdam: Elsevier Scientific Publishers.
15. Hu, W. J., Woychik, N., and Chu, F. S (1983). ELISA of picogram quantities of aflatoxin Ml in urine and milk. J. Food Prot., 47:126-127.
16. Groopman JD (1994). Molecular dosimetry methods for assessing human aflatoxin exposures. In The Toxicology of Aflatoxins: Human Health,

Veterinary and Agricultural Significance, pp. 259–276 [DL Eaton and JD Groopman, editors]. New York: Academic Press.

17. Zhu JQ, Zhang LS, Hu X, Xiao Y, Chen JS, Xu YC, Fremy J & Chu FS (1987).Correlation of dietary aflatoxin-B1 levels with excretion of aflatoxin-M1 in human-urine. *Cancer Research;* 47**,** 1848–1852.
18. WHO (2006). Child growth standards based on length/height and age. *Acta paedr Suppl*;450:76-85
19. Qian, Geng-Sun *et al.*(1994). A Follow-up Study of Urinary Markers of Aflatoxin Exposure and Liver Cancer Risk in Shangai, People's Republic of China. *Cancer Epidemiology, Biomarkers and Prevention*; 3: 3- 10.
20. Victora CG, de Onis M, Hallal PC, Blössner M, Shrimpton R (2010). Worldwide timing of growth faltering: revisiting implications for interventions using the World Health Organization growth standards. *Pediatrics*; 125(3):e473-480
21. Groopman JD, Johnson D, Kensler TW (2005). Aflatoxin and hepatitis B virus biomarkers: A paradigm for complex environmental exposures and cancer risk. *Cancer Biomark*; 1: 5-14.
22. Turner PC. Collinson AC. Cheung YB. Gong YY. Hall AJ. Prentice AM. Wild PC (2007). Aflatoxin exposure in utero causes growth faltering in Gambian infants *Int. J. Epidemiol*; 36: 1119-1125.
23. Turner PC, Sylla A, Gong YY, Diallo MS, Sutcliffe AE, Hall AJ, Wild CP (2005). Reduction in exposure to carcinogenic aflatoxins by postharvest

intervention measures in West Africa: a community-based intervention study. *Lancet* ; 365: 1950–56.

CHAPTER 5

ENVIRONMENTAL EXPOSURES AND IMPLICATIONS FOR CHILD HEALTH AND GROWTH

Observational data discussed in chapter one indicates that children take chicken feces, soil or stones into their mouths. In addition, 17 % of the 287 households we studied Zimbabwe were exposed to substantial levels of aflatoxin. The design and implementation of optimal WASH and food safety interventions need to consider these critical environmental exposures that could synergistically act to overcome the intestinal lining barrier function leading to endotoxemia and a cascade of energy expensive immune stimulation processes that divert energy from growth (1).

Aflatoxin M1 is a transient marker of dietary exposure to aflatoxin B1. It shows exposure to aflatoxin in the last 2 to 3 days prior to urine sample collection. However, research in West African has suggested that households exposed to aflatoxin are likely to be frequently exposed over time with seasonal variations (2). It is therefore likely that exposed households would be chronically exposed over time, since poor post-harvest practices and storage facilities are not likely to change over time given the generally low awareness of this public health problem among small scale holder farmers. Aflatoxin is anti-nutritive and inhibits protein synthesis and metabolism of multiple micronutrients (3). Protein synthesis inhibition may alter gut intestinal architecture, intestinal regeneration, impair tight junction formation; which together reduce intestinal barrier function and subsequent translocation of macromolecules and antigens into systemic circulation, systemic immune activation, processes which may lead to

impaired growth (4). In addition, reduction in intestinal surface area may reduce zinc absorption.

Systematic assessment of WASH as exposure assessment of fecal bacteria has not been explored comprehensively with growth indicators as the outcomes. Most literature in this area has looked at water and sanitation and whenever hygiene is involved it has been defined as hand washing and / or health education. A composite measure of personal, indoor and outdoor environment hygiene has not been looked at and most studies have reported effects on growth based on water and sanitation spot checks which are subject to information bias if assessed at one time point in a cross-sectional study. The aggregate measure of hygiene developed in chapter 3 is one of the strengths of this study.

WASH related assets have been included in the socio-economic index in most of the studies in the past, thus making it difficult to understand WASH and nutritional outcomes relationships while adjusting for socioeconomic status. With the recent hypothesis (5) of the importance of fecal bacterial overload might play in etiology of environmental enteropathy, optimal WASH indicators for this exposure assessment need to be well defined. In this study, we report that an environmental hygiene index is a good measure and a significant predictor of linear growth faltering in over 2 year old children in rural Ethiopia. After adjusting for most of the confounders known for WASH-HAZ association, and including IYCF in the model, the hygiene index remained significant and independent of IYCF, SES and recent morbidity. These results concur with the earlier studies that showed diarrhea may not be an important predictor of linear growth faltering in the long term. The independent effect of environmental hygiene

emphasizes the need to focus on hygiene independent of SES and IYCF, and target interventions to each of these domains in order to gain an additive benefit from programs and interventions.

Quantitative methods need to be complemented with in-depth behavior observation for better understanding of the interaction between infants and children and their environment. In rural Ethiopia, mothers reported children do not play out in the yard. An in-depth observation would have confirmed such self-reports and helped to explain the lack of association seen for water and sanitation variables in most age groups. Rural Ethiopia (SNNPR) is remarkably different from rural Zimbabwe. In the southern region, large animals are usually tethered in closed proximity (just besides the main house or kitchen, **Figure 5.1)** and in some cases even inside the kitchen. The households in SNNPR do not have a clearly trimmed yard that is easy to sweep clean, and it is unlikely the infants can be out on their own. Large animals were common in these households and poultry were rare. However, the contrary is true for rural Zimbabwe.

Households in rural Zimbabwe had clearly defined yards that were easy to sweep. Large animal corrals were far away from the yard, though the yards were not fenced and poultry, which were common, roamed freely into the yard and inside the kitchen even during infant feeding **(Figure 5.2).** In addition, active infants between 6-18 months crawled on bare soils which were frequently contaminated with animal feces, even though the feces were not always visibly evident. Rural Zimbabwean households had bare loose and coarse soils which dried up relatively quickly after rains, compared to soils in SNNPR which were fine, wet and sticky and mixed with large animal feces.

In most households, the lack of a clearly defined yard made it hard to define any place that the mother and child could take cover due to overgrown grass in some households. These contrasts between two impoverished, rural African settings with high infant stunting rates illustrate the necessity that context be well evaluated while planning a WASH intervention.

.

Figure 5.1: Rural Ethiopia- SNNPR

Feeding young children in rural Zimbabwe, 2010. M Mbuya

Figure 5.2: Rural Zimbabwe

Conclusions and recommendations for research

Infants' play and feeding environment was frequently contaminated with fecal indicator bacteria in rural Zimbabwe as objectively confirmed by microbiological methods. WASH interventions strategies should target infants environment in a context specific way. Such interventions should address clear separation of chicken (and other animals) feces from baby's environment and protect infants from ingesting earth and chicken feces. Context differences in infants' play and feeding environment were clear for rural Zimbabwe and Ethiopia. An additive environmental hygiene index is a more useful indicator of overall hygiene and measurement of exposure compared to single spot checks for animal and human feces. In assessing presence of fecal contamination in the household yard, a single time point observation is limited, and therefore we recommend in-depth qualitative assessment to complement cross sectional data.

The effect of WASH on linear growth in 2-5 year old children, independent of SES and IYCF, emphasizes the need to target improvement of WASH in addition to IYCF, because both may have additive effects in preventing growth failure. Our results suggest that environmental hygiene accounts for more variation in height for age Z score than the WHO IYCF indicators. Although this might reflect poor accuracy of the ICYF indicators, our finding underscores the need to focus more on hygiene interventions. Hygiene education should complement efforts in hand washing with elements that focus on cleaning the household environment to which the infant is exposed. As much as community led total sanitation program (CLTS) has succeeded in promoting construction of latrines and safe human feces disposal, animal feces disposal remains inadequate. The makeshift latrines observed in SNNPR were not safe for young

children and safe disposal of children feces might be inadequate. Post-harvest interventions discussed in chapter 3 would also add to the health benefits and eventually improve growth by reducing chronic exposure to aflatoxin.

Future research is recommended to understand the mechanisms by which aflatoxin exposure is linked to growth failure. Assessing chronic aflatoxin exposure (aflatoxin albumin adduct) and the markers of environmental enteropathy (EE) can elucidate the mechanisms underlying growth failure. A longitudinal study would show trends over time in infant health and growth that arise from exposure to aflatoxin, and would establish temporality which our cross-sectional study could not. Further, co-exposure to other mycotoxins, for example, fumonisin and deoxynivalenol, should be assessed and their relevance to gut health toxicity elucidated. Geospatial and seasonal distribution of these contaminants of staples should be studied further in Sub-Saharan Africa to provide evidence for targeting interventions.

Further mechanisms to elucidate the role of fecal bacterial overload of the gut are critical to confirm the underlying hypothesis that linear growth faltering is primarily mediated through environmental enteropathy (EE). Microbial tracking using specific molecular techniques could identify the relative risk from animal and human feces in EE etiology. Molecular characterization techniques hold potential in identifying the source, distribution and fate of fecal bacteria in household environment and inform intervention strategies to minimize such contamination.

Our results raise the need for a greater understanding of the contextual risk factors for stunting in rural Ethiopia. The significant association with the hygiene index notwithstanding, minimal variation in growth outcomes was accounted for by our

measures of WASH and infant feeding. More attention should be directed to understanding the risk factors for poor growth *in utero* in Ethiopia. In-depth observation studies to understand the interaction of infants with their surrounding environment will identify priorities for WASH interventions targeted to infants. Community Led Total Sanitation program should focus on both animal and human feces disposal and the planning of household environment and animal corrals for easy maintenance of cleanliness. Lastly, the role of water usage (quantity), storage and water quality in predicting poor nutrition outcomes should be studied further in this context.

REFERENCES

1. Campbell DI, Elia M, Lunn PG (2003). Growth faltering in rural Gambian infants is associated with impaired small intestinal barrier function, leading to endotoxemia and systemic inflammation. *J Nutr*; 133:1332–8.
2. Gong YY, Hounsa A, Egal S, Turner PC, Sutcliffe AE, Hall AJ, Cardwell K, Wild CP (2004). Postweaning exposure to aflatoxin results in impaired child growth: A longitudinal study in Benin, West Africa. *Environ Health Perspect;*112:1334–8.
3. Williams JH, Phillips TD, Jolly PE, Stiles JK, Jolly CM, Aggarwal D (2004). Human aflatoxicosis in developing countries: A review of toxicology, exposure, potential health consequences, and interventions. *Am J Clin Nutr*; 80:1106–22.
4. Smith LE, Stoltzfus RJ, Prendergast A (2012). Food chain mycotoxin cxposure, gut health, and impaired growth: a conceptual framework. *Adv. Nutr.*, 3: 1–6.
5. Humphrey JH (2009). Child undernutrition, tropical enteropathy, toilets, and hand washing. *Lancet*, 374: 1032—1035.