STUNTING SPATIAL PATTERN IN RWANDA

Vestine UWIRINGIYIMANA

STUNTING SPATIAL PATTERN IN RWANDA

AN EXAMINATION OF COMPLEMENTARY FEEDING PRACTICES, MYCOTOXINS EXPOSURE AND ENVIRONMENTAL FACTORS

DISSERTATION

to obtain
the degree of doctor at the University of Twente,
on the authority of the rector magnificus,
prof.dr. T.T.M. Palstra,
on account of the decision of the Doctorate Board,
to be publicly defended
on Wednesday 20th November 2019 at 16:45 hrs

by

Vestine UWIRINGIYIMANA

born on 20th July 1985

in Kampala, Uganda

This thesis has been approved by
Prof.dr ir. A. Veldkamp, supervisor
Dr. S. Amer, co-supervisor

ITC dissertation number 371
ITC, P.O. Box 217, 7500 AE Enschede, The Netherlands

ISBN 978-90-365-4894-6
DOI 10.3990/1.9789036548946

Cover designed by Job Duim
Printed by ITC Printing Department

UNIVERSITY OF TWENTE. | ITC

Graduation committee:

Chairman/Secretary

Dean of the Faculty	University of Twente

Supervisor

Prof.dr.ir. A. Veldkamp	University of Twente

Co-supervisor(s)

Dr. S. Amer

Members

Prof.dr.ir. A. Stein	University of Twente
Prof.dr. V.G. Jetten	University of Twente
Prof.dr.mr. C. Lachat	University of Gent
Dr. A. Melse	Wageningen University

Dedicated to my mum

Acknowledgements

First and foremost, I acknowledge God for having enabled me to start and finish well my PhD journey. If it weren't through faith in God and his grace to carry on, I wouldn't have made it.

Secondly, my sincere acknowledgement and gratitude go to Nuffic NICHE project in collaboration with the Center for Geographical Information and Science (CGIS) of the University of Rwanda, who provided me with this opportunity to do this PhD. I extend my appreciation to the University of Rwanda, through and the College of Agriculture, Animal Sciences and Veterinary Medicine and the Department of Food Science and Technology for facilitating me to further my studies. I want to acknowledge the institutions that in one way or the other assisted us in making this research possible. My sincere gratitude goes to the School of Public Health through the Department of Human Nutrition, College of Medicine and Health Sciences of the University Rwanda that provided us portable height boards and the electronic scales for anthropometric measurements of children and their caregivers. Besides, I thank the Ministry of Health, the Rwanda Biomedical Center and the Rwanda Agricultural Board for providing useful data and information for this research.

My most profound appreciation goes to my promoter, Prof. Dr. Tom Veldkamp for his wise guidance, his continuous support, patience and encouragement throughout my thesis journey. I have a deepest respect and gratitude for you, as you never lacked time for our meetings even though, as the Dean, your agenda was full. Your immense knowledge and broad scientific perspective helped me to conduct my PhD successfully. I would also like to thank Dr Sherif Amer, my daily supervisor, you made my PhD journey smooth by making sure I had all I needed to succeed. Thank you for your constant encouragement, scientific and logistic support, and for critically reviewing my work. I would also like to thank Dr Marga Ocke, from whom her expertise in nutrition assessment made the first part of this research possible. I am grateful for your support in the processing of the 24-hour dietary recall data, your critical comments and revision of my work that led to the first two publications of this thesis. I would also like to thank Dr Frank Osei, for bringing in your expertise in geostatistics. Thank you for taking the time and working with us on this relevant and engaging topic of stunting in children. I want to extend my gratitude Dr Alphonse Nkurunziza, who very much encouraged me to apply for this PhD, and now I am successfully at the finish line. I am grateful to the ITC faculty and the PGM department. Special thanks go to Loes Colenbrander, your assistance since the start of my PhD till the thesis publication stage; to Petra Weber, thank

you not only for the administrative support but also for being a source of inspiration to keep a healthy soul in a healthy body. To Ing. Frans van den Bosch, thank you for your technical support during my PhD.

We extend our appreciation to the caregivers who participated in this study, together with their children. We also thank the local authorities which who permitted us to collect the data in Musanze and the interviewers who visited each household to administer the questionnaire and collect anthropometric measurements. I would also like to thank my fellow ITC PhD colleagues from Rwanda, Dr Elias Nyandwi, Dr Adrie Mukashema, Alice Nikuze and Marc Manyifika, and all the past MSc students from Rwanda at ITC. Your company at ITC and your moral support, wise counsel and encouragement helped me to cope with the pressure that comes from being a PhD student. Thank you for all the good times we had in ITC and doing extracurricular activities together which made the pressure of a PhD melt away. Dr Elias and Marc, thank you also for assisting me in getting the spatial data I needed for this research. I want to thank my colleagues and also former classmates at the Department of Food Science and Technology for your encouragement. Special thanks also go to Peter Mugisha and Mediatrice Uwanyirigira, for your support and advice during my fieldwork time in Rwanda. My sincere thanks also go to Silver Karumba, Pacifique Mukashema, Dr Eric Matsiko, for their assistance in getting the secondary data I considered to use in my research. I appreciate the support of Damien Iyakaramye, Dr Maryse Umugwaneza and Jean de Dieu Habimana for their advice and assistance during my first fieldwork. Also, I am extremely grateful to my friends Henriette, Chantal, Joselyne, Nicole, Eseosa, Atinuke, Joanah and Donald, for their constant encouragement, spiritual and moral support during my PhD journey.

I want to express my heartfelt gratitude to my mum, to whom I dedicate this thesis. I thank her for her unwavering support during my career, for her advice in my young age to follow sciences even when I wasn't confident enough to select the sciences option for my high school education. You instilled in me that I was capable, you built in me the confidence I needed, and you had seen the light in me even before I could see it myself. And from high school to graduating with honours at my Bachelors, obtaining an MSc degree, and now graduating with a PhD degree. Thank you, mum, for your unwavering support, your prayers and your advice that sparked all the light in me. Choosing sciences was the best decision I have ever made for my career, I loved it since day one, and along my journey as a scientist my passion grew even more. You are the best mum anyone could desire to have, and I am incredibly grateful for your guidance! I also want to extend my appreciation to my close family

Gonzag, Adeline, Alain, Claire, Christelle and Beata for your moral and spiritual support since the start of my PhD journey and in my life in general.

Last but not least, I am grateful to my husband Johannes; meeting you and getting to know you while doing my PhD and getting married to you has been the highlight of my PhD journey. Your company during the last two years of my PhD made my journey all the more worthwhile; thank you for your understanding, your encouragement, and for your prayers.

Table of Contents

List of figures

List of tables

Chapter 1. General Introduction

1.1 Background on stunting

Stunting or linear growth retardation is a widespread global problem especially in developing countries. Worldwide, it is estimated that 150.8 million children (22.2%) of children less than five years are stunted (Development Initiatives, 2018). Africa and Asia have the highest number of stunted pre-school children with an estimate of 58 million and 87 million respectively (FAO et al., 2017). However, Africa has the highest stunting prevalence of 30.3% compared to Asia which has 23.2% (Development Initiatives, 2018).

Stunting occurs when a child is not growing in height in accordance with his/her potential (Stewart et al., 2013). *Linear growth retardation or impaired linear growth* are other terms used for stunting. Stunting is the result of multiple circumstances and determinants, including antenatal, intra-uterine and postnatal malnutrition (de Onis et al., 2012). Stunting is defined as the proportion of children whose height-per-age falls below -2SD of the Z-score of the WHO reference population (de Onis et al., 2006; Stewart et al., 2013).

Evidence from 54 low- and middle-income countries indicates that growth faltering generally begins during pregnancy and continues to about 24 months of age (UNICEF, 2013). The period from the start of a pregnancy until a child is two years old is termed as the "1000 days window of opportunity". This 1,000-day period is a critical time for structural brain development; therefore pregnant and breastfeeding mothers who cannot access the right nutrients are more likely to have children with compromised brain development and who suffer from poor cognitive performance (McDonald & Thorne-Lyman, 2017). Almost half of the growth retardation that occurs in children takes place during the complementary feeding period (Stewart et al., 2013). First, this is

because growth is highest in early childhood, which means that the nutritional requirements are also high. Because children have limited gastric capacity, they require energy and nutrient dense foods as a complement to breast milk. Second, infections during early childhood counteract growth as they are more severe in pre-school children and can thus reduce appetite and limit the absorption of essential nutrients. Third, as young children are totally dependent on their caretaker for nourishment, they can easily be prone to poor care practices (Martorell et al., 1994).

Determinants of stunting

In 2013, the World Health Organization (WHO) released an updated conceptual framework on childhood stunting in the context of complementary feeding (Stewart et al., 2013). This new framework was based on the UNICEF (1990) conceptual framework for malnutrition and provided a solid, in-depth overview of the determinants of stunting. The causes of stunting were classified by WHO into four proximal factors: household and family factors, inadequate complementary feeding, breastfeeding practices and infection (Stewart et al., 2013). Firstly, the household and family factors comprise the maternal factors such as the preconceptional conditions of a child and the home environment in which the child lives. Different elements in the home environment can affect a child's growth such as inadequate care practices and low caregiver education. Secondly, inadequate complementary feeding involves poor food quality, inappropriate feeding practices, and poor food and water safety all of which contribute to stunted growth and development. Thirdly, inadequate breastfeeding practices such as non-exclusive breastfeeding exposes a child to stunting through nutrient deficiencies and low immunity. Lastly, the presence of chronic infection such as diarrhoeal disease, helminth infection and malaria can severely affect child growth and development through chronic inflammation and nutrient sequestration or loss.

Stunting results in increased morbidity and mortality in affected children and leads to poor cognition, motor and language development. Also, its economic consequences involve increased health expenditures and opportunity costs incurred in caring for the sick child. In the long run, stunting leads to reduced educational performance, low adult wages, loss of work capacity and productivity, increased risk of chronic diseases and short adult stature. The latter can have implications for pregnancy outcomes as it leads to increased risk of maternal mortality and short- and long term disability due to obstructed labour (Martorell, 1999; de Onis et al., 2012). The long term consequences of stunting often lead to a downward spiral of stunting from generation to generation; mainly because a stunted mother is more likely to have a stunted baby (ACC/SCN & IFPRI). Therefore, to adequately address stunting, not only children less than two years should be the focus but also women of childbearing age, and pregnant and lactating mothers. To tackle the problem of chronic malnutrition at its roots, causes of stunting should be addressed in the context of community and societal factors in which stunting occurs. This is because stunting is a multifactorial problem; therefore nutrition interventions alone cannot provide the solution, but a multisectoral approach is required to sustainably prevent stunting.

Spatial heterogeneity of stunting

Stunting, which is measured at the individual level, is inherently spatially variable within communities and regions. Prevalence is usually reported on a national or sub-national level which overshadows the spatial heterogeneity in stunting that exists at lower administrative levels or finer geographical scale within countries. The mapping of child growth failure in Africa (Osgood-Zimmerman et al., 2018a) showed that although stunting has reduced overall, there are geographical differences in stunting prevalence across the African continent. Precision public health, which is the use of granular data to efficiently target interventions to populations most in need for the efficient use of

resources, is still a challenge in Sub-Saharan Africa (Dowell et al., 2016). This is mostly because data is often lacking and where available, it is not spatially detailed enough to be used for research or for decision-making (Marx et al., 2014). Thus, policies and interventions implemented on a local level that use national or sub-national estimates could lead in some instances, to resources not being properly targeted to the most vulnerable (Osgood-Zimmerman et al., 2018b). Thus, the lack of geographic data on a detailed spatial scale is still a major limitation for an in-depth assessment of stunting and its drivers (Marx et al., 2014). With the current rate of stunting reduction, if the spatial variation in stunting is not addressed to set targeted interventions, there will be likely no country in Africa to achieve the sustainable development goals (SDG) targets in all its territory, despites improvement in national-level stunting estimates (Osgood-Zimmerman et al., 2018a). Thus, considering the spatial variability of stunting is imperative, if the goals to reduce stunting on a longer term are to be reached.

In most dietary surveys, the spatial component is not considered; and when taken into account, the sampling conducted does not take into account the spatial component. To respond to the increased need for data for spatially detailed evidence-based decision making, the Demographic Health Surveys (DHS) Program provides georeferenced data of household clusters in the more recent population-based surveys since 2014 (Gething et al., 2015). The georeferenced data can be used to study the determinants of stunting on a more spatially detailed scale, target interventions to the most vulnerable, and measure progress towards health and nutrition goals. Although the DHS provide spatially detailed data, the survey takes place only every five years. Thus, to efficiently monitor the nutritional status of children, nutrition surveys conducted to inform policy or monitor program implementations need to include the spatial component both in the sampling design and data analysis.

In the recent years, the application of spatial analysis methods using Geographical Information System (GIS) to analyse and predict stunting has been gaining momentum. Particularly, model-based geostatistical methods have been applied in analysing and predicting stunting on a detailed spatial scale in countries such as Nigeria, Kenya and Tanzania (Bosco et al., 2017) and Ethiopia (Hagos et al., 2017). Model-based geostatistics, which makes inferences from spatially correlated phenomena, offer a great advantage of examining the risk factors of stunting by taking into account the spatial dependency in the outcome, predicting stunting on a detailed spatial scale, and studying unexplained residual stunting not accounted for by specified models. In turn, the results obtained can be used to inform policy and allow for spatially targeted interventions.

Stunting situation in Rwanda

The latest 2015 Rwanda Demographic Health Survey (RDHS) showed that 38% of children under five in Rwanda are stunted (NISR et al., 2015). This is a severe situation as any stunting prevalence beyond 30% is considered 'very high' (de Onis et al., 2019). The East-Africa region is particularly affected by high levels of stunting (36.7%), compared to the rest of the continent (FAO et al., 2017). The stunting prevalence in Middle Africa is 32.5%, Western Africa 31.4%, Southern Africa 28.1% and Northern Africa 17.6% (FAO et al., 2017).

Rwanda has made progress in reducing the prevalence of acute malnutrition, infection rate and increasing food security nationally. Despite these achievements, unacceptable food consumption and the rate of chronic malnutrition remain high. The negative impact of the high stunting levels is not only felt by the affected children and families but it also has implications on a national level. The 'Cost of Hunger study' conducted in Rwanda in 2013 (AUC & NEPAD, 2013) revealed that there were more stunted children in Rwanda than 10 years before,

only 1 out of every 3 children with undernutrition was estimated to be receiving proper health attention, and more health costs associated with undernutrition occurred before the child turned 1 year old. Furthermore, 21.9% of all child mortality cases in Rwanda were associated with undernutrition, 12.7% of all repetitions in primary school were associated with stunting, and as a consequence stunted children achieved 1.1 years less in school education than non-stunted children. For the impact of stunting on adult productivity, the 'Cost of Hunger study' showed that child mortality associated with undernutrition had reduced the Rwandan workforce by 9.4%. Secondly, 49.2% (3 million) of the adult population in Rwanda aged 15-64 years had suffered from stunting as children, and lastly, the annual costs associated with child undernutrition were estimated at 503.6 billion RWF, equivalent to 11.5% of Gross Domestic Product (GDP) (AUC & NEPAD, 2013). Therefore, due to its effect on the different sectors of the country, multisectorial approaches are needed to alleviate chronic malnutrition in Rwanda.

To intensify efforts in the fight against malnutrition, Rwanda introduced the National Food and Nutrition Policy (NFNP) in 2013. The policy came along with the National Food and Nutrition Strategic Plan 2013-2018 and was an update of both the 2007 country's first National Nutrition Policy (NNP) (MOH, 2005) and the 2010-2013 National Multisectorial Strategy to Eliminate Malnutrition (NmSEM). The policy was updated to better reflect the multifactorial facet of chronic child malnutrition in Rwanda (MINALOC et al., 2013). Additionally, the policy was aligned with the country's Second Economic Development and Poverty Reduction Strategy (EDPRS II) in which food security and malnutrition had been made a foundation issue (GoR, 2013). As part of the National Food and Nutrition Strategic Plan, Rwanda launched a 1000 days nutrition campaign that focused on behaviour and social change communication to improve the nutrition of children at the community level (MOH, 2013). The target set in the strategic plan was to reduce

stunting from 44% to 24.5% on a national level by 2018 (MINALOC et al., 2014b). However, the gap between the target and the current prevalence is still large.

Policies implemented in the previous years have helped to see a reduction in stunting levels from 44% in 2010 (DHS, 2012) to 38% in 2015 (NISR et al., 2015). However, the disparity in stunting levels between the different districts of the country is very pronounced. In the recent Rwanda Strategic Review of Food and Nutrition Security (MIGEPROF, 2018), it was acknowledged that much effort and scientific evidence are still needed to understand the specific drivers of stunting in some regions of the country.

1.2 Rationale of the thesis

In Rwanda, the need for more scientific evidence of the drivers of stunting is justified by the high geographical disparity in prevalence levels across the country. A survey conducted in 2010 by the World Food Programme (WFP et al., 2012), showed that the Northern region of Rwanda was extremely affected by stunting (figure 1). Overall, regions with high rates of unacceptable food consumption also had the highest stunting levels particularly the Western province. Contrarily, the Northern volcanic region of Rwanda that had low rates of unacceptable food consumption[1] had the highest levels of stunting. Thus, this pronounced geographic variation in stunting prevalence called for more in-depth research on the drivers of stunting in Rwanda.

[1] The combination of poor and borderline food consumption, which represent consumption of staples and vegetables less than one day per week, and a daily consumption of staples and vegetables with a frequent (4 days/week) consumption of oil and pulses, respectively.

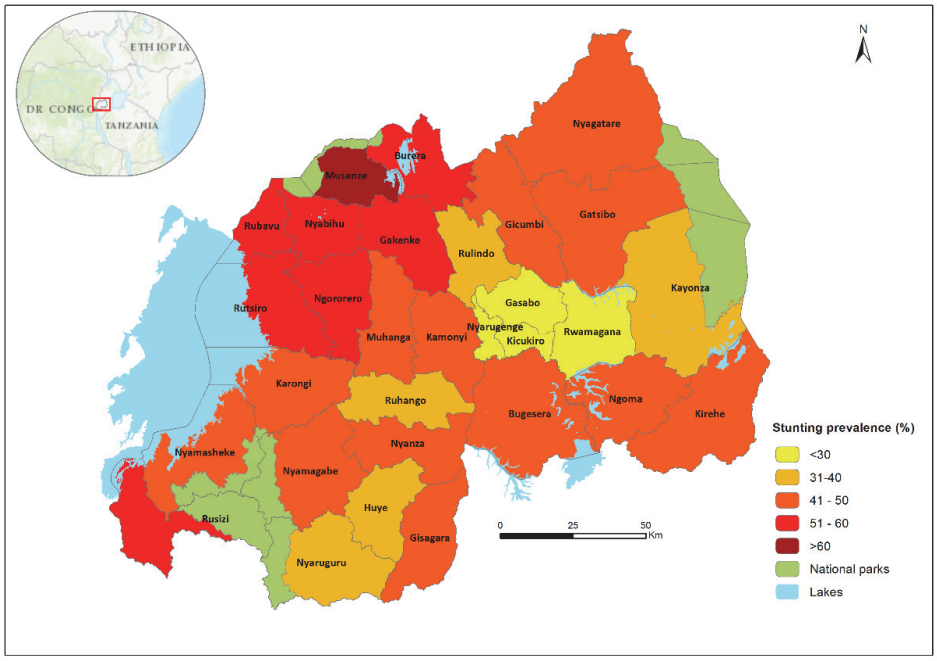


Figure 1. Distribution of stunting per district in Rwanda (Source: CFSVA, 2012)

As stated in the determinants of stunting section, inadequate complementary feeding practices are among the drivers of stunting in children. In Rwanda, there is a gap in the scientific evidence on the quality and the safety of the complementary foods fed to the growing children. For diet quality, micronutrient deficiencies in children are still a challenge in many parts of the developing world. Anaemia as a result of iron deficiency and its effect on undernutrition status of children has received more attention in the past (NISR et al., 2015; Kateera et al., 2015). Zinc deficiency which is known to be associated with stunting in children, hasn't been well investigated in the Rwandan context. Additionally, the safety of complementary foods requires similar attention. Mycotoxins especially aflatoxins exposed to during the complementary period are known to negatively affect the linear growth of children. Although exposure starts in utero, the exposure to aflatoxins increases considerably during the weaning period (Gong et al., 2004).

Thus, evidence is lacking concerning the micronutrient quality and the safety of the complementary diet of children in Rwanda. As stunting is a result of multiple causes, it is also imperative to look beyond nutrition. Thus, environmental determinants of stunting also require attention in light of their influence both on dietary zinc intake and mycotoxins exposure in children. To this date, there has not been any research focusing on elucidating the effect of dietary zinc intake and mycotoxins exposure on stunting in Rwanda. The influence of zinc deficiency, aflatoxins exposure and environmental factors on linear growth in children is discussed in the section below.

Inadequate dietary zinc intake and stunting

Zinc is among the micronutrients often lacking in plant-based complementary diets of children in developing countries. Zinc is one of the most abundant elements in human cells with 95% of the body zinc found within the cells. It is required for the activity of more than 100 enzymes involved in major metabolic pathways in the body and is therefore essential for a wide range of biochemical, immunological and clinical functions (Gibson, 2006; King, 2011). These functions include but are not limited to DNA[2] and RNA[3] metabolism, protein synthesis, gene expression, cell growth and differentiation and cell-mediated immunity (Lowe et al., 2009). Therefore, zinc is especially important during the period of rapid growth, both pre- and postnatal, and for tissues with rapid cellular differentiation and turnover such as the immune system and the gastrointestinal tract (Brown et al., 2001).

Zinc deficiency develops from inadequate dietary intake of zinc-rich source foods, and low zinc bioavailability from foods due to high antinutrient content of plant-based diets. Moreover, zinc deficiency may

[2] DNA: Deoxyribonucleic acid. It is a molecule that encodes the genetic instructions in every living organism
[3] RNA: Ribonucleic acid. It serves in the regulation and expression of the genetic information stored in DNA

also develop from decreased absorption, excessive losses, increased requirements in pregnancy/lactation and during a period of stress, tissue anabolism and rapid growth (Nriagu, 2018). Gibson found that the three leading causes of zinc deficiency in developing countries are dietary factors (low intake and poor availability of dietary zinc), excessive losses and high physiological requirements (Gibson, 1994). Most of the diets in developing countries and Rwanda particularly, are plant-based with almost no animal source foods such as meat, poultry and fish (Brown et al., 2001). Animal source foods are a rich source of zinc as it is readily available for absorption. On the contrary, diets based on starchy roots and cereals, are known to either be low in zinc for starchy foods or high in phytic acid for cereals (Kaur et al., 2014). Phytic acid is an antinutrient component of cereals known to inhibit the absorption of zinc. On the other hand, excessive losses of zinc may occur mainly through diarrhoea and parasitic infections such as malaria, hookworm and schistosomiasis (Gibson, 1994).

There is a large body of evidence of the effect of zinc on linear growth in children. Zinc deficiency is associated with stunted growth, impaired immunity and poor weight gain in children (Ferguson et al., 1993; Brown et al., 2001; Salgueiro et al., 2002; Gibson, 2006; Lowe et al., 2009). Children less than five years and pregnant women are the most affected by zinc deficiency because of their increased physiological needs (Gibson, 2006). Although severe zinc deficiency is rare, mild zinc deficiency in childhood is common (Ferguson et al., 1993; Gibson, 1994). Not only does zinc deficiency causes impaired linear growth in children, it also causes impairments in body composition, taste acuity, appetite, and delays in sexual maturation. Reviews of the effect of zinc supplementation in children showed that preventive zinc supplementation has a significant effect on the linear growth in children (Bhutta et al., 2008; Brown et al., 2009; Mayo-Wilson et al., 2014). On the other hand, height-for-age, a measure of stunting, is the best known

factor to measure the adverse outcomes associated with zinc deficiency in populations (Engle-Stone & Hess, 2007). This is because growth failure is the most prominent clinical feature of mild zinc deficiency. The WHO considers zinc deficiency to be a public health problem when the prevalence of stunting among children less than five years of age is more than 20% (Hotz & Brown, 2004; Engle-Stone & Hess, 2007). Thus, a prevalence of stunting of 38% shows that zinc deficiency in Rwandan children is most likely widespread. Also, Rwanda has been placed under the countries which are at high risk of zinc deficiency (Wessells & Brown, 2012). Every country with a prevalence of stunting higher than 25% and which has optimum zinc intake less than 15% was classified as being at a higher risk. In this category, Rwanda ranked 11 out of 182 countries worldwide by descending risk of zinc deficiency (Wessells & Brown, 2012). Currently, no research has been done in Rwanda to evaluate the effect of inadequate dietary zinc intake on stunting.

Zinc deficiency can also be measured through assessment of serum zinc concentration or assessment of inadequate dietary intake. The level of zinc in blood plasma or serum is the best available biomarker of risk of zinc deficiency in populations (Engle-Stone & Hess, 2007; Brown, 2012). Additionally, dietary zinc intake measurement using a quantitative dietary survey is used to evaluate the extent of zinc deficiency. This is important because the primary cause of zinc deficiency is a chronic inadequate dietary intake of bioavailable forms of zinc (Gibson, 2007). Due to the high levels of stunting in Rwanda and given that no study has been done yet to evaluate the complementary feeding practices focusing on dietary zinc intake, the investigation of dietary zinc intake levels among children is warranted.

Aflatoxins and their effect on linear growth

Mycotoxins are toxic to humans and animals. Although many species of these toxigenic moulds have been identified, only a few of them,

particularly those affecting cereals such maize, wheat, barley and groundnuts, are considered to be significant for humans (FAO, 1991). The most important mycotoxins include aflatoxins, fumonisins, deoxynivalenol, zearalenone, and ochratoxins (Gnonlonfin et al., 2013). Particularly, aflatoxins are the most foodborne mycotoxins and have the greatest impact on human health in tropical countries (WHO, 2003). Exposure to aflatoxins can cause both acute and chronic toxicities that range from death to toxic effects on the central nervous system, cardiovascular and pulmonary systems and the gastrointestinal tract (FAO, 2001). Aflatoxins are toxic secondary metabolites of the fungi *Aspergillus* species mainly the *Aspergillus flavus* and *A. parasiticus*. *Aspergillus* species are more widely associated with food commodities during drying and storage (Pitt, 2000).

In developing countries, especially in Sub-Saharan Africa, aflatoxins exposure has been linked to the development of liver cancer (Liu & Wu, 2010). Also, exposure to aflatoxins among infants and young children results in linear growth retardation (Khlangwiset et al., 2011). Although the exposure to aflatoxins starts early in the pre-natal stage, it increases rapidly during and after the weaning period (Gong et al., 2004). It is with no doubt that many people, especially children are constantly exposed to high levels of aflatoxins in Sub-Saharan Africa (Strosnider et al., 2006), and Rwanda is not spared. This is primarily because of the favourable climatic conditions for the Aspergillus fungi to grow on maize and peanuts, and produce aflatoxins. Moreover, many countries in Africa still lack the appropriate mechanisms to control the aflatoxins contamination in the local food chain, as the local supply of commodities is still largely informal and thus not well regulated (Gbashi et al., 2018). Consequently, aflatoxins are widespread in major dietary staples in Africa (Wagacha & Muthomi, 2008). Maize and groundnuts are the primary vehicles for aflatoxins contamination with a lesser occurrence in sorghum, yam and cassava. In Rwanda, high levels of

aflatoxins were found in food commodities with maize and peanuts being the highest contaminated commodities which had concentrations exceeding the acceptable range. Quantities of aflatoxins found in maize, peanuts and cassava had mean levels of 892.3µg/kg, 661.5µg/kg and 114.3µg/kg respectively (Nyinawabali et al., 2013). These levels far exceeded the acceptable limit of 10µg/kg for total aflatoxins set by the Rwanda Standards Bureau (RSB, 2012).

The prevention and control of aflatoxins contamination require the implementation of good agricultural practices (GAP) during the pre-harvest and post-harvest period which can include the use of physical, chemical and biological treatments (WHO, 2006a). The pre-harvest methods consist of the use of resistant varieties, use of biological and chemical agents, and field and harvest management. The post-harvest methods can include improved drying methods, good storage conditions and the use of natural and chemical agents (Adegoke & Letum, 2013).

The analysis of aflatoxins contamination in complementary foods is done by assessing aflatoxins contamination in foods used during complementary feeding for example maize and peanuts (Egal et al., 2005; Khlangwiset et al., 2011). The foods can be weaning flour samples collected directly from households. By analysing the complementary flours consumed by children and identifying the amount of flour children consume per day, the level of aflatoxins to which children are exposed can be quantified. The commonly used methods for laboratory analysis of aflatoxins are the immune-chromatographic assays such as lateral flow strips; the quantification of mycotoxins using a fluorescence densimeter (Egal et al., 2005); the use of High Performance Liquid Chromatography (HPLC) (Abdulkadar et al., 2004; Nyinawabali et al., 2013); and the use Liquid Chromatography-Mass Spectrometry (LC-MS) (Shephard, 2008a). The use of immune-chromatographic assays has been

on the rise since the past decade (Dzantiev et al., 2014). These are rapid test methods of detection and quantification of mycotoxins in various food matrices. Compared to the traditional methods, they offer an advantage as they are less time consuming, enable appropriate monitoring for food safety thereby facilitating trade and the protection of human and animal health. (Li et al., 2014). This is especially beneficial for developing countries, as the gold standard methods such as LC-MS are often lacking or unfordable. Although there has been some research done on the African continent on the effect of aflatoxins and fumonisins on linear growth in children, such a study has not yet been conducted in Rwanda. Therefore, this research will also elucidate the impact of dietary mycotoxins exposure in children to their height-for-age as a measure of stunting prevalence.

Influence of environmental factors on diet quality and the application of GIS

Environmental factors are known to influence the availability, dietary quality and safety of foods. Therefore, as part of the multifactorial approach in understanding stunting in Rwanda, the influence of different environmental factors is worth to be assessed. From their effect on food security, environmental factors influence directly or indirectly the occurrence of chronic malnutrition in communities. Different studies have been conducted in Kenya (Grace et al., 2012), Mali (Jankowska et al., 2012), in Mexico (Skoufias & Vinha, 2012) and Nigeria (Rabassa et al., 2014). Grace et al. (2012) analysed the correlation between surface temperatures and rainfall levels on the rates of stunting in Kenya. They found out that as warming and drying continued to increase in different parts of the country due to climate change, the rate of malnutrition would also continue to rise.

Apart from influencing child malnutrition in general, environmental factors also have a direct effect on the occurrence of mycotoxins.

Mycotoxins production is influenced by extrinsic environmental factors such as temperature, humidity, and oxygen level; and intrinsic factors to the crop such as moisture content, water activity, substrate and plant type, and insect infestation (Gnonlonfin et al., 2013; Adegoke & Letum, 2013). Bunyavanich et al. (2003) showed that higher temperatures and extreme weather conditions encourage the growth of mycotoxin-producing fungi. Also, the alternation of drought and flooding contribute to the mycotoxins production because the drought weakens the seed kernels of plants allowing the fungal contamination, while flooding or periods of increased humidity causes moist conditions perfect for fungal growth.

The analysis of the impact of environmental factors on child stunting will be done through the application of statistical and spatial analysis using geographic information systems (GIS). GIS is defined as a computer-based information system that enables capture, modelling, manipulation, retrieval, analysis and presentation of geographically referenced data (Worboys, 2003). GIS consists of data models, structures for storing the data, the data itself, software for query, retrieval, analysis and mapping; and the hardware used to support these functions (Cope & Elwood, 2009). The application of GIS offers a significant advantage to the traditional way of visualising data in spreadsheets as data is viewed on a map which may reveal patterns or connections that are not easily seen in tables or text (ESRI, 2012). On the other hand, presenting nutrition data spatially can, in turn, allow for more effective communication amongst stakeholders and consequently, facilitate decision-making. The application of GIS in nutrition research is a developing field, as GIS is usually applied in public health especially in disease mapping (Ricketts, 2003; Maheswaran & Craglia, 2004). Thus, this research will not only add up to the scientific knowledge base in Rwanda, but it will also enrich the scientific field in general in terms of the application of GIS in the nutrition field.

The conceptual framework of this research adapted from the WHO framework on stunting is summarised in figure 2. Environmental factors and household factors are at the base of the framework as the contextual factors associated with stunting. From contextual factors, there are proximal factors grouped under the child and maternal factors, and the complementary feeding practices. The usual child and maternal factors known to be drivers of stunting are taken into account to study the influence of poor complementary diet quality in terms of inadequate dietary zinc intake and consumption of contaminated foods that lead to mycotoxins exposure.

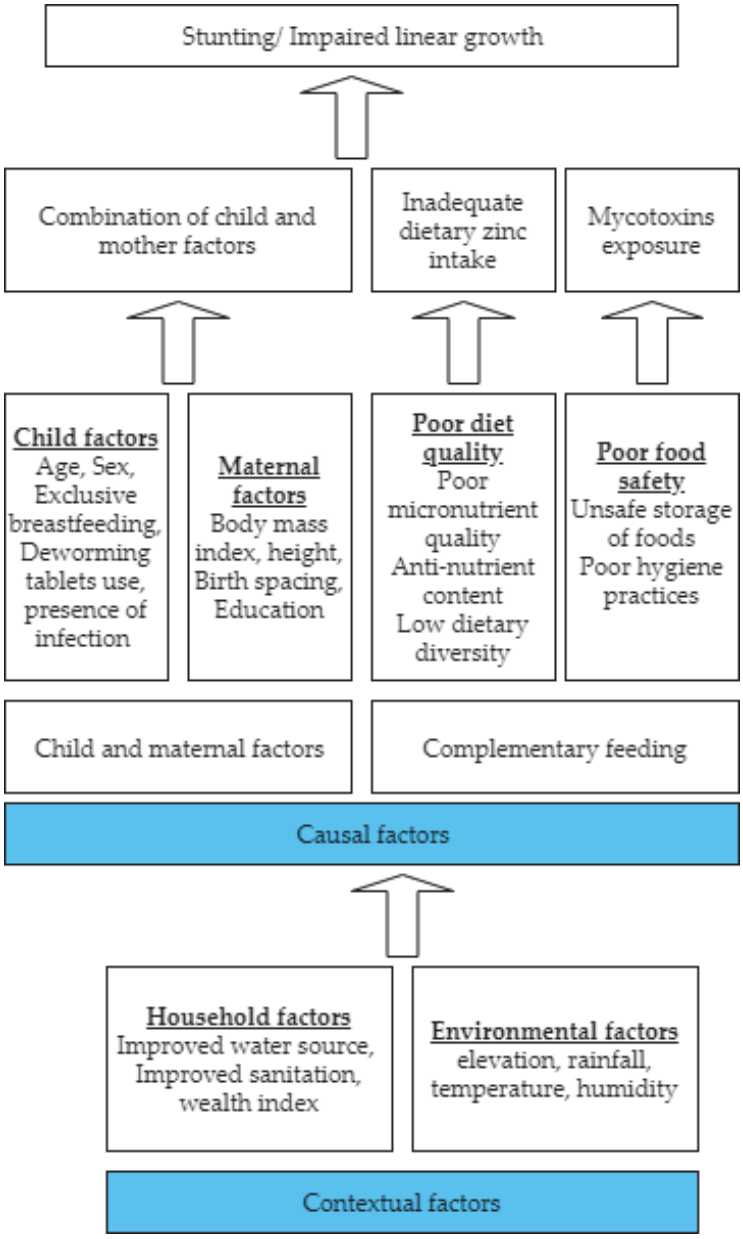


Figure 2. Conceptual framework of this research

In order to understand the drivers of stunting in Rwanda, this research was conducted on a two scale level. The first part focused on conducting a local case-study to understand the determinants of stunting by

focusing on complementary feeding practices and mycotoxins exposure in the Northern Province of Rwanda, in the district of Musanze. The second part involved up-scaling the analysis to a national level by applying GIS for mapping and spatially modelling of stunting prevalence. The objectives of the thesis are outlined below.

1.3 Objectives of the thesis

The overall aim of this research is to study the determinants of stunting in relation to complementary feeding practices, poor diet quality, mycotoxins exposure and environmental factors. To achieve the study aim, the research has the following specific objectives:

1. To study the determinants of stunting in the Northern region of Rwanda with particular focus on the complementary feeding practices and dietary zinc intake
2. To determine the aflatoxins level content of complementary flours namely maize and peanut, estimate the exposure to aflatoxins in children and determine its relationship to stunting observed in the same study population
3. To carry out a spatially disaggregated analysis of stunting determinants by taking into account socio-economic factors biophysical factors and mycotoxins contamination.
4. To model the spatial pattern of stunting in Rwanda using a Bayesian geostatistical model and estimate spatial residual effects for the identification of possible mycotoxins influence on stunting on a national level

This research hypothesises that "complementary feeding practices and mycotoxins exposure contribute significantly to the stunting burden in Rwanda".

1.4 Description of study area

Rwanda is located in Eastern Africa between latitude 1°04′ and 2°51′ South and longitude 28°50′ and 30°50′ East and is boarded by Uganda, Tanzania, DRC and Burundi. Rwanda covers 26,338 km² with a population of nearly 11 million among which 1.5 million are under five years, and only 17% of the whole population is urban (NISR & MINECOFIN, 2012). Administratively, Rwanda has five provinces, 30 districts, 416 sectors and 2148 cells. Figure 3 shows the map of the country divided into the provinces and the districts. The district is the basic political-administrative unit of the country and Kigali is the capital. Rwanda's topography is characterised by high mountains to the West, rolling hills in the centre, and savanna, plains and swamps to the East (NISR & MINECOFIN, 2012). Thus, the altitude of the country varies from 900 meters to 4500 meters and the highest point is Mount Karisimbi with 4507 meters. Due to its topography, Rwanda has a moderate tropical climate with temperatures averaging 20°C and an average yearly rainfall of 2400 mm (Mendelsohn et al., 2016). Agriculture and the service sectors are the two main drivers of the Rwandan economy (MINAGRI et al., 2016).

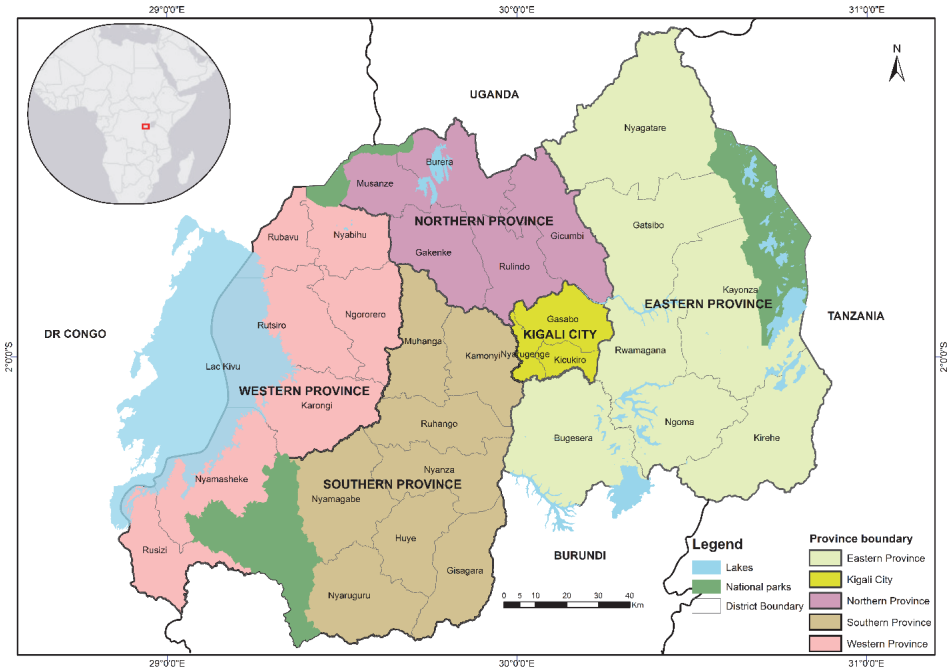


Figure 3. Administrative map of Rwanda

1.5 Outline of the thesis

The thesis consists of six chapters, structured in four sub-studies according to the specific research objectives. **Chapter 1** provides the scientific background information of the study, states the research problem, research objectives and describes the study area. **Chapter 2** presents the predictors of stunting in children in the Northern region of Rwanda. In this chapter, complementary feeding practices with dietary zinc intake, and socio-economic determinants are examined. In **Chapter 3**, aflatoxin contamination levels in complementary flours collected in households visited in Chapter 2 are measured. The association of aflatoxins exposure from complementary feeding flours to stunting is assessed. Chapter 3 leads to the nationwide analysis of stunting determinants using the latest Rwanda DHS data in **Chapter 4**. The contribution of socio-economic factors, complementary feeding practice and environmental factors to stunting are shown. Also, the chapter shows the impact of having access to markets at the lower end of food

supply chain as a proxy for mycotoxins exposure on stunting. In **Chapter 5**, high resolution spatial mapping of stunting using a Bayesian geostatistical model is conducted. To conclude, in **Chapter 6** a synthesis of the main findings through the methods applied is provided as well as a reflection on the thesis findings. The implications for policy and future research are also discussed.

Chapter 2. Predictors of stunting with particular focus on complementary feeding practices: A cross-sectional study in the Northern Province of Rwanda[4]

[4] This chapter is based on a published paper: Uwiringiyimana, V., Veldkamp, A., Ocké, M. C., & Amer, S. (2018). Predictors of stunting with particular focus on complementary feeding practices: A cross-sectional study in the Northern Province of Rwanda. Nutrition. doi:10.1016/j.nut.2018.07.016

Abstract

Objective: The aim of this study was to review the factors associated with stunting in the northern province of Rwanda by assessing anthropometric status, dietary intake, and overall complementary feeding practices.

Methods: A cross-sectional study with 138 children aged 5 to 30 mo was conducted. A structured questionnaire was used to collect information on socio-demographics of each mother and child, breastfeeding and complementary feeding practices. Anthropometric status was assessed using height-for-age z-scores for children and body mass index for caregivers. Dietary intakes were estimated using a 24-hour recall. Multiple linear and logistic regression models were performed to study the predictors of height-for-age z-scores and stunting.

Results: The stunting prevalence was 42%. Prevalence of continued breastfeeding and exclusive breastfeeding were 92% and 50% respectively. Most children (62%) fell into the low dietary diversity score group. The nutrient intake from complementary foods was below recommendations. The odds of stunting were higher in children >12 mo of age (Odds ratio [OR] = 1.18; 95% CI 1.08-1.29). Exclusive breastfeeding (OR= 0.22, 95% CI 0.10-0.48) and deworming tablet use in the previous 6 mo (OR= 0.25; 95% CI 0.07-0.80) decreased significantly the odds of stunting in children. Also, the BMI of caretaker (β=0.08 kg/m2; 95% CI 0.00-0.17) and dietary zinc intake (β= 1.89 mg/d; 95% CI 0.29-3.49) were positively associated with the height-for-age z-scores.

Conclusion: Interventions focusing on optimal nutrition during the complementary feeding stage, exclusive breastfeeding and the use of deworming tablets have the potential to substantially reduce stunting in children in the Northern Province of Rwanda.

***Keywords*:** stunting, dietary intake, complementary feeding practices, exclusive breastfeeding, deworming tablets, children, Rwanda

2.1 Introduction

Stunting also termed linear growth retardation, occurs when a child is not growing in length or height in accordance with his or her potential (Stewart et al., 2013). Globally, about 22.9% of children aged less than five years are stunted (FAO et al., 2017). Africa and Asia have the highest numbers of stunted children estimated at 59 million and 87 million respectively (FAO et al., 2017). Nationally, 38% of children under five in Rwanda are stunted (NISR et al., 2015). WHO considers stunting to be a public health problem when the prevalence of stunting among children less than five years of age is higher than 20% (Lander et al., 2010). Growth retardation begins during pregnancy and continues until two years of age (Victora et al., 2010). Almost half of the growth retardation happens during the complementary feeding period (Dewey & Huffman, 2009).

The WHO framework provides an overview of the causes of stunting and classifies them into four main proximal factors: household and family factors, inadequate complementary feeding practices, inadequate breastfeeding practices, and infection (Stewart et al., 2013). In practice, multicausality is usually present which makes stunting one of the most difficult health challenges to address. For example, the problem of infection and its impact on child health is worsened when zinc deficiency is present. Zinc deficiency has been associated with stunted growth, impaired immunity and poor weight gain in children (Brown et al., 2001; Salgueiro et al., 2002; Gibson, 2006). Inadequate dietary zinc intake in its bioavailable forms is the most likely cause of zinc deficiency (Gibson, 2007).

Rwanda has successfully managed to reduce the prevalence of wasting or acute malnutrition in children to 2% (NISR et al., 2015). However, the reduction in stunting is limited despite the efforts to reduce its prevalence(MINALOC et al., 2014a). Thus, there is a need for scientific research to assess the locally relevant predictors of stunting. Previous studies in Rwanda have focused more on the socio-demographic factors,

child health care, and parasite infection in children and their influence on undernutrition or stunting prevalence (Mupfasoni et al., 2009; Binagwaho, Condo, et al., 2014; Lu et al., 2016). As far as we know, this is the first study to combine complementary feeding practices and nutrient intake assessed through the 24-hour recall, to study the predictors of stunting in Rwanda and Musanze District particularly.

2.2 Methods

Study overview

A cross-sectional study was conducted in May 2015 in Musanze District which has a high stunting prevalence of 38% (NISR et al., 2015). Most of the population in the district live in the rural area. The study population consisted of children aged 5 to 30 mo and their caregivers. A required sample size of 145 children was estimated taking into account the estimation of mean dietary zinc intake based on previous studies (Ferguson et al., 1993; Gewa et al., 2007; Berti et al., 2011) considering a power of 80%, a significance level of 0.05 and a non-response rate of 10%. Cluster-random sampling was applied using villages in Musanze District as the sampling frame and households as the basic sampling units. Five villages out of thirty-eight were randomly selected, and a random walk method (UN, 2005) was used to visit the households in each sector. All households with a child aged 5 to 30 mo had an equal chance of being asked to participate in the survey. No caregiver refused to participate in the study.

Ethical approval

Ethical approval was obtained from the Institutional Review Board of the College of Medicine and Health Sciences in Rwanda. Permission to collect data in Musanze was also obtained from local authorities. Participants signed an informed consent form after the research aim and objectives were explained to them.

Interactive 24-hour recall

An interactive and multi-pass 24-hour recall questionnaire, adapted and validated for use in developing countries, was used (Gibson & Ferguson, 2008) (Appendix 2). The questionnaire applied a multi-pass method in which the first pass consisted of gathering a list of foods consumed the previous day. The second pass consisted of probing for more information about the food consumed such as time of the day, food specification and the cooking method used. The third pass consisted of estimating the portion sizes using local household utensils, units or monetary values and recording the ingredients of the homemade mixed dishes consumed by the child. The fourth and final pass consisted of reviewing the recall information to ensure the accuracy of the data gathered. For the administration of the questionnaire, graduated food models were assembled and calibrated; five qualified interviewers were trained, and a pilot test was done. The food intake data was assessed through a single 24-hour recall with the caregiver of each child as the respondent, and at study population level, each day of the week was included. The 24-hour recall questionnaire also included a yes/no question to know if the food the child ate the previous day was similar to his or her usual food intake.

Data processing of the food intake data was done in Excel 2010. The estimation of energy and nutrient intake from the 24-hour recall was done by compiling a local food composition database using nutrient composition from published sources (Murphy et al., 2004; Lukmanji et al., 2008; Stadlmayr et al., 2012; USDA, 2015). The food matching was done following the guidelines published by (FAO/INFOODS), and Greenfield and Southgate (2003). The Murphy model to estimate the intake of available zinc was applied where the zinc availability factor was set to 0.10 if the phytates-to-zinc ratio was greater than 30; 0.15 for ratios between 15 and 30; and 0.30 for ratios less than 15 (Gibson & Ferguson, 2008). To assess the quality of the complementary diets of

children, a dietary diversity score (DDS) was calculated for which each of the seven food groups consumed received a score of one. A DDS of ≥4 was classified as high dietary diversity, whereas a DDS <4 was classified as low dietary diversity (WHO, 2010).

Household questionnaire and anthropometric measurement

The household questionnaire was adapted from the validated Rwanda Demographic and Health Survey household questionnaire (NISR et al., 2015). It comprised questions on the socio-demographic characteristics of mother and child, household characteristics, breastfeeding and complementary feeding practices, and child current and past illness. Socio-demographic characteristics included age, gender, marital status, education, and employment. Household characteristics included household size, wealth category, drinking water source, water treatment before use and access to agricultural land. Breastfeeding and complementary feeding practices included exclusive breastfeeding in the first 6 mo, continued breastfeeding, vitamin A supplementation in the previous 6 mo, deworming tablets use in previous 6 mo and micronutrient powder use. Child illness included the presence of diarrhoea, cough, malaria, and flu in the previous 4 wk and presence of illness the previous day to the interview.

Anthropometric measures of children and their respective caregivers were recorded. Birth weight and child age were obtained from parental recall or the child's birth certificate. The height of children was measured in recumbent position using a height board designed by UNICEF and was recorded to the nearest 0.1 cm. The height of caregivers was measured in the standing position without shoes to the nearest 0.1 cm using a portable stadiometer. The weight of both caregiver and child was measured in duplicate to the nearest 0.1 kg using an electronic scale (SECA Model 803, Hanover, MD, USA) (WHO, 1995). The WHO *Anthro software* version 3.2.2 (WHO, 2011) was used to

calculate the height-for-age z-scores (HAZ), the weight-for-age z-scores (WAZ) and the weight-for-height z-scores (WHZ). According to WHO criteria, a Z-score of <-2 for HAZ indicates stunting; for WAZ, undernutrition; and for WHZ, wasting. For descriptive purposes, further classifications of height-for-age as adequate (-2<HAZ< +2), moderately stunted (-3< HAZ< -2) and severely stunted (HAZ< -3) were used (WFP & CDC, 2005). Extreme values for HAZ, WAZ, and WHZ were (-6, +6), (-6, +5) and (-5, +5) respectively; these were automatically flagged in Anthro software, and in subsequent data analysis, they were considered as outliers. For caregivers, BMI was classified as mild undernutrition (16≤BMI<18.5 kg/m^2), normal (18.5<BMI≤24.9 kg/m^2), overweight (25.0<BMI≤29.9 kg/m^2) and obese (BMI≥30.0 kg/m^2) (WFP & CDC, 2005). For comparison between age groups, the age of children (months) was split into four age groups: 5-11 mo, 12-17 mo, 18-23 mo and 24-30 mo.

Statistical analysis

Continuous variables were checked for normality and log-transformation was conducted if needed. Frequencies and percentages were reported for categorical variables, and means (SD) or medians (interquartile range) were reported for continuous variables. Spearman's rank order correlation was used to study the bivariate association between variables. For group means or percentage comparison between stunted and non-stunted children, independent sample *t* test or Pearson χ^2 test were used. Multiple linear regression was used to study the association between HAZ and the explanatory variables. The explanatory variables were from the socio-economic characteristics of mothers and children, household characteristics, breastfeeding and complementary feeding practices, and child illness status. A backward linear regression model was conducted on all predictors, and the predictors in the last model were fitted in a linear regression model together with energy and zinc intake variables. Interaction factors of age groups and energy intake were also tested as the energy intake can

differ within age groups of children. The adjusted R^2 was reported for model cross-validation. Similarly, a logistic regression model was fitted to the data with the binary indicator of stunting as the dependent variable to obtain odds ratios (OR) and 95% confidence intervals (CIs). The model Nagelkerke R^2 was reported. Multicollinearity was checked using Pearson pairwise correlation coefficient and variance inflation factor (VIF) statistic, with $r > 0.7$ and VIF > 0.5 as cut-off values for the indication of multicollinearity in the regression model (Vatcheva et al., 2016). Consequently, the energy intake and the interaction factor of age group 18-23 mo and energy intake that introduced multicollinearity, were not considered in the model. A model sensitivity analysis with the linear regression model was tested by including only children whose intake in the previous day was similar to their usual food intake. For all the analyses, a *p*-value <0.05 indicated statistical significance. All statistical analyses were performed using SPSS version 24 (IBM, Armonk, NY, USA).

2.3 Results

Study participants

In total, 145 infants and their caregivers participated in the study with 67 (46%) boys and 78 (54%) girls. For seven children, there were missing values on HAZ, and the data of these children were excluded for the present analyses. The child, caregiver and household characteristics are shown in Table 1. Most of the caregivers were mothers (95%) of the children. The majority of caregivers (67%) had a primary education; whereas 22% were illiterate. The mean age of caregivers were 28 ± 8 y; 73% of caregivers had a normal BMI, whereas 3% were mildly undernourished, 20% were overweight and 4% were obese. The mean household size was 4.7 ± 1.8 persons. Of the households, 34% and 58% were in the first (lowest) and second wealth category, respectively. One-third of households (34%) had a kitchen garden, 73% had access to agricultural land, and 38% had livestock.

Table 1. Child, caregiver and household characteristics by stunting status of children between 5 to 30 mo (n=138) in Musanze District, Rwanda

	Non-stunted (n=77)	Stunted (n=61)	Total	
Characteristic	N (%) or mean ±SD			*p-value**
Sex child				
Girls	44 (57)	28 (46)	72 (52)	0.189
Boys	33 (43)	33 (54)	66 (48)	
Children age groups				
5-11 mo	34 (44)	14 (23)	48 (35)	0.021
12-17 mo	25 (32)	19 (31)	44 (32)	
18-23 mo	13 (17)	19 (31)	32 (23)	
24-30 mo	5 (7)	9 (15)	14 (10)	
Relationship with child				NA
Mother	71 (93)	59 (97)	130 (95)	
Other	5 (7)	2 (3)	7 (5)	
Caregiver education				0.152
Illiterate	13 (17)	17 (28)	30 (22)	
Primary level	52 (68)	40 (66)	92 (67)	
Secondary & tertiary level	11 (15)	4 (6)	15 (11)	
Caregiver marital status				NA
Married (monogamy)	66 (87)	53 (87)	119 (87)	
Married (polygamy)	3 (4)	4 (7)	7 (5)	
Unmarried	7 (9)	4 (6)	11 (8)	
Caregiver age (y)	28.3 ±7.5	28.3 ± 9.1	28.4 ± 8.2	0.992†
Caregiver height (cm)	159 ± 5.8	159 ± 5.4	159 ± 5.7	0.777†
BMI of caregiver				NA
Mild undernutrition	2 (3)	2 (3)	4 (3)	
Normal	51 (68)	48 (79)	99 (73)	
Overweight	19 (25)	9 (15)	28 (20)	
Obese	3 (4)	2 (3)	5 (4)	
Household size	4.8 ± 1.8	5.0 ± 1.9	4.7 ± 1.8	0.507†
Wealth category of household				0.770
First (lowest) category	27 (35)	20 (33)	47 (34)	
Second category	44 (58)	35 (57)	79 (58)	
Third category	5 (7)	6 (10)	11 (8)	
Kitchen garden-Yes	30 (40)	16 (26)	46 (34)	0.103†
Access to agricultural land-Yes	56 (74)	44 (72)	100 (73)	0.839
Livestock ownership-Yes	32 (42)	20 (33)	52 (38)	0.264

BMI, body mass index; N/A, n was too low for statistical testing.

**P*-value: Two-sided, obtained through Pearson χ^2.

†Independent sample t test was performed.

Anthropometric results

Figure 4 shows the growth curve of the study population as compared to the WHO standard growth curve. The overall mean (SD) was -1.58 (1.77), -0.86 (1.31) and 0.22 (1.32) for HAZ, WAZ and WHZ respectively. In total, 44% of children were stunted, among which 62% were moderately stunted and 38% were severely stunted. Also, among stunted children, 54% were boys and 46% were girls. Undernutrition prevalence was 16%, of which 22% was severely undernourished. Wasting prevalence was 7%, of which 39% was severely wasted (Uwiringiyimana et al., 2018a).

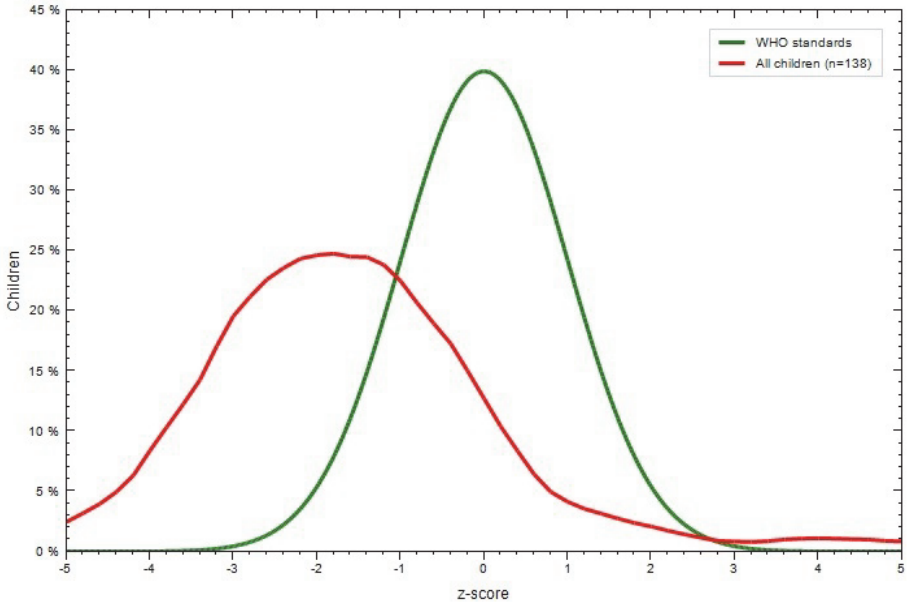


Figure 4. Height-for-age z-score distribution of children aged 5 to 30 mo of age (n=138) in Musanze District compared to the WHO Standard Curve

Child feeding practices

Child feeding practices are shown in Table 2. Among the non-stunted children, exclusive breastfeeding in the first 6 mo of their life was 66% compared to 31% in the stunted children ($p<0.001$). Although not significant, differences between pre-weaning age groups for stunted and non-stunted, age groups at first introduction of complementary foods

and the presence of diarrhoea, cough, and flu in the previous 4 wk were observed. The majority of all children were still breastfeeding (92%) and most of the children (65%) who received pre-weaning foods were in the age range of 4-5 mo. The reason for feeding children before they turn 6 mo old was mainly that the child wanted to eat (34%), was sick (26%), or had colic disease (18%). Traditional herbal mixture (29%), fruit juice (24%), plain water (18%) and porridge (16%) were the most commonly used pre-weaning foods. For weaned children (8%), 55% were in the 13-24 mo age group, and 36% of children were in the 7-12 mo age group (Uwiringiyimana et al., 2018a). The majority of the children were introduced to complementary meals around the age of 6 to 9 mo (72%). The use of vitamin A supplements in the previous 6 mo was 93%; whereas the use of micronutrient powder in children's diet in the previous 4 wk was 38%. Of all children, 73% had received deworming tablets in the previous 6 mo, and 37% of children had been ill the previous day. The presence of diarrhoea (34%), vomiting (14%), malaria (9%), and flu (33%) in the previous 4 wk was low as compared to coughing (72%).

Table 2. Description of breastfeeding, complementary feeding practices, presence of illness (presence of infection) and food group consumption per non-stunted and stunted children (5-30 mo) in Musanze District, Rwanda

		Non-stunted	Stunted	Total	
		N (%)			*p*-value*
Breastfeeding practices					
Exclusive breastfeeding	Yes	50 (66)	19 (31)	69 (50)	<0.001
Current breastfeeding	Yes	68 (91)	57 (93)	125 (92)	NA
Breastfeeding frequency					
2-3 times/d		6 (9)	4 (7)	10 (8)	NA
>3 times/d		60 (91)	50 (93)	110 (92)	
Complementary feeding practices					
Pre-weaning age groups					
1-3 mo		10 (38)	14 (33)	24 (35)	0.86
4-5 mo		16 (62)	28 (67)	44 (65)	
Age groups at first introduction of complementary foods					
1-5 mo		17 (23)	21 (35)	38 (28)	0.17
6-9 mo		57 (77)	39 (65)	96 (72)	
Vitamin A supplements in previous 6 mo	Yes	71 (93)	57 (93)	128 (93)	NA
Micronutrient powder (MNP) use in previous 4 wk	Yes	30 (40)	22 (36)	52 (38)	0.81
Illness (or presence of infection)					
Deworming tablets use in previous 6 mo	Yes	55 (72)	44 (73)	99 (73)	1
Diarrhoea in previous 4 wk	Yes	23 (30)	23 (38)	46 (34)	0.46
Vomiting in previous 4 wk	Yes	9 (12)	10 (16)	19 (14)	0.6
Malaria in t previous 4 wk	Yes	7 (9)	5 (8)	12 (9)	1
Cough in previous 4 wk	Yes	57 (75)	41 (67)	98 (72)	0.41
Flu in previous 4 wk	Yes	28 (37)	17 (28)	45 (33)	0.35
Previous day illness	Yes	27 (36)	23 (38)	50 (37)	0.93
Food groups consumption					
Grain, roots & tubers	Yes	73 (95)	60 (98)	133 (96)	NA
Legumes & nuts	Yes	59 (77)	50 (82)	109 (79)	0.54
Dairy products (milk, yogurt, cheese)	Yes	3 (4)	0 (0)	3 (2)	NA
Flesh foods (meat, fish, poultry & liver/organ meats)	Yes	7 (9)	4 (7)	11 (8)	NA
Eggs	Yes	1 (1)	2 (3)	3 (2)	NA
Vitamin A rich fruits & vegetables	Yes	61 (79)	43 (71)	104 (75)	0.32
Other fruits & vegetables	Yes	40 (52)	25 (41)	65 (47)	0.26
Dietary diversity score (DDS)					
Average score, mean (SD)		3.2 (1.1)	3.0 (1.1)	3.1 (1.1)	0.41†
Low DDS (<4 food groups)		47 (61)	38 (62)	85 (62)	1.00

DDS, dietary diversity score; N/A, if n was too low for statistical testing in the group for non-breastfed children, for children, who did not receive vitamin A, and for those that did not consume the specific food group. **P*-value: Two-sided, obtained by Pearson χ^2. †Independent sample t test was performed.

The main staple foods consumed in Musanze were sorghum, maize, potatoes, beans and green leafy vegetables. Consequently, the most consumed food groups on the recall day were grain, roots, and tubers (96%), legumes and nuts (79%), and vitamin A rich fruits and vegetables (75%) (Table 2). Animal source foods were the least consumed by both non-stunted and stunted children; with dairy products, flesh foods and eggs consumed by 2%, 8% and 2% of children respectively. The mean (±SD) dietary diversity score among the study population was 3.1±1.1. The majority of children (62%) had consumed food from less than four food groups and thus were in the low dietary diversity group. There was no significant difference between DDS and stunting status (Table 2), and the consumption of specific food groups by children was similar across all age groups (Uwiringiyimana et al., 2018a).

Quantification of nutrient intake

Intakes of energy and nutrients from complementary foods are shown in Table 3. Considering average breast-milk intake per age group, the median energy intake was low as compared to the energy required from complementary foods. The same was observed for macronutrients such as protein, fat, and carbohydrates. It should, however, be noted that these requirements were set for total intake including breastfeeding. Assuming low bioavailability, zinc intakes were also low compared to requirements across age groups. The zinc, iron, vitamin A, and vitamin C intakes included intakes from micronutrient powder, but only one caregiver had included it in the meal of her child the day before the interview. The main food groups that contributed to energy and nutrient intake for all children were cereals, vegetables, and fats and oils.

HAZ and stunting predictors

From the multiple linear regression analysis, age groups, exclusive breastfeeding, use of deworming tablets, body mass index of caregiver, dietary zinc intake were predictors of HAZ (Table 4).

Table 3. Predictors of height-for-age z-scores in 135 children aged 5 to 30 mo in Musanze District, Rwanda (adjusted R^2=0.27)

Variables	β	*P*-value	95% CI for β	
			Lower bound	Upper bound
Age (mo)				
Age group 12-17mo vs 5-11mo	-1.08	0.034	-2.08	-0.08
Age group 18-23mo vs 5-11mo	-2.27	<0.001	-3.19	-1.35
Age group 24-30mo vs 5-11mo	-2.14	0.002	-3.49	-0.79
Exclusive breastfeeding (yes)	0.76	0.006	0.22	1.29
Deworming tablets use in the previous 6 mo (yes)	1.99	<0.001	1.16	2.83
BMI of caregiver (kg/m2)	0.08	0.049	0.00	0.17
Dietary zinc intake (mg)	1.89	0.021	0.29	3.49
Interaction terms between age groups and energy intake				
Age group 12-17mo*energy intake	-0.002	0.049	-0.004	0.000
Age group 24-30mo*energy intake	-0.003	0.040	-0.005	0.000

BMI, body mass index.

The model adjusted R^2 was 0.27. By comparing age groups, children who were in the higher age groups were more likely to be stunted than children in the 5-11 mo age group. Also, exclusive breastfeeding together with the use of deworming tablets, a higher BMI of caregiver and a higher dietary zinc intake positively predicted height-for-age in children.

Table 4. Dietary intake of energy and nutrients from complementary foods per age groups in children between 5 to 30 mo of age in Musanze District, in comparison to requirements (based on 24-hour recall method)

	Age groups, mo									
	5-11 (n=49)			12-17 (n=46)		18-23 (n=35)		24-30 (n=14)		
Nutrient	Median	25th, 75th	EAR (RNI)	Median	25th, 75th	Median	25th, 75th	Median	25th, 75th	EAR (RNI)
Energy (kcal/d)*	107	65, 332	**417**	202	91, 345	282	141, 415	247	84, 426	**772**
Protein (g/d)	3	1, 9	**(11)**	6	3, 9	8	4, 13	7	2, 14	**(13)**
Fat (g/d)	2	1, 5	**30†**	2	1, 6	4	1, 6	2	0, 4	**30-40‡**
Carbohydrate (g/d)	19	10, 49	**95†**	35	18, 62	52	29, 76	51	18, 87	**100**
Iron (mg/d)§	0.9	0.5, 1.4	**(18.6)**	1.3	0.7, 2.1	2.2	1.1, 2.9	2	0.5, 4.1	**11.6**
Calcium (mg/d)	19	7, 42	**(400)**	23	11, 49	42	24, 65	30	5, 45	**417**
Magnesium (mg/d)	29	17, 58	**(54)**	42	25 75	74	40, 103	76	21, 133	**(60)**
Vitamin A (µg/d)	6	2, 27	**286**	14	1, 41	32	2, 95	1	0, 36	**286**
Vitamin C (mg/d)	6	2, 13	**(25)**	6	3, 15	10	6, 16	8	2, 13	**25**
Zinc (mg/d)‖	0	0.0, 0.1	**4**	0.1	0.0, 0.1	0.1	0.1, 0.2	0.1	0.0, 0.2	**2**

AI, adequate intake; AMDR, acceptable macronutrient distribution range; EAR, estimated average requirement; RNI, recommended nutrient intake. 25th, 75th, interquartile range. Otherwise indicated, RNI values were taken from (WHO/FAO), EAR values are from Allen et al. (2006) and RDA values for protein from (IOM, 2002/2005).

*Energy required from complementary foods assuming average breast-milk energy intake (Dewey & Brown, 2003). (For the age group 5-11mo, energy required was estimated as an average between requirements for age groups 6-8mo [356kcal/d] and 9-11mo [479kcal/d]).

†Adequate intake (IOM, 2006).

‡AMDR (Acceptable Macronutrient Distribution Range) is the range of intake for a particular energy source that is associated with reduced risk of chronic disease while providing intakes of essential nutrients (IOM, 2002/2005).

§Iron: assuming a 5% bioavailability (Allen et al., 2006).

‖Zinc: assuming low bioavailability from unrefined, cereal-based diet (IZiNCG, 2004).

There was no significant association between energy intake and stunting; however, when age was taken into account, energy intake inversely predicted height-for-age in children aged 12-17 mo (β= -0.002; 95% CI, -0.004-0.000) and 24-30 mo (β= -0.003; 95% CI, -0.005-0.000). From the model sensitivity analysis limited to 116 children for which intake on the recalled day was similar to their usual intake, all the variables significantly predicted height-for-age except the age group 12-17 mo (β= -0.92; 95% CI, -7.55- -3.10), dietary zinc intake (β= 1.13; 95% CI, -0.52- 2.79), and the interaction factors (Uwiringiyimana et al., 2018a).

For the estimation of risk of stunting in children using logistic regression analysis (Table 5), as the child grew older by 1 mo, the odds of stunting increased by 20% (OR=1.18; 95% CI, 1.08-1.29). On the other hand, the odds of being stunted were significantly lower if a child had been exclusively breastfed (OR 0.22; 95% CI, 0.10-0.48) and had received deworming tablets in the previous 6 mo (OR 0.25; 95% CI, 0.07-0.80). The model Nagelkerke R^2 was 0.29.

Table 5. Predictors of risk of stunting in children between 5 and 30 mo (n=136) in Musanze District, Rwanda

Variables	OR	*p*-value	95% CI for OR	
			Lower bound	Upper bound
Age (mo)	1.18	<0.001	1.08	1.29
Exclusive breastfeeding (yes)	0.22	<0.001	0.10	0.48
Deworming tablets use in the previous 6 mo (yes)	0.25	0.02	0.07	0.80

R^2=0.29 (Nagelkerke).

2.4 Discussion

Stunting prevalence (44%) in the study population was higher compared to the general prevalence (38%) reported for the District of Musanze. We examined the predictors of height-for-age z-scores and stunting in our study population. Children aged 12-17 mo, 18-24 mo and 24-30 mo were

more likely to be affected by stunting as compared to those aged 5-11 mo. This confirms the increase in stunting observed during the complementary feeding period. As observed by Dewey and Huffman (Dewey & Huffman, 2009), a combination of factors such as low birth length, lack of exclusive breastfeeding in the previous 6 mo suboptimal complementary feeding and presence of infection, exposes older children to stunting. In our study, we believe the lower exclusive breastfeeding rate and the low quality of complementary foods could be playing a role. In rural Rwanda, similar results were found where being older than 12 mo was a risk factor for stunting (Ngirabega et al., 2010). Both exclusive breastfeeding and the use of deworming tablets in the previous 6 mo were independently associated with a lower risk of stunting in children. Exclusive breastfeeding is known to provide all essential nutrients for growth and immunity of a child within the first 6 mo of life thus offering a protective effect against stunting (WHO, 2001). Although we did not find a significant association between continued breastfeeding and height-for-age, the former has been shown to improve linear growth in mostly deprived children (Onyango et al., 1999). Infection that translates into persistent diarrhoea negatively affects a child's development and growth, whereas malnutrition predisposes a child to infection (Checkley, Buckley, Gilman, Assis, Guerrant, Morris, Molbak, et al., 2008). In the present study, the use of deworming tablets was associated with significantly lower odds of stunting in children, although we did not find an association with infections. In Southern Rwanda, Heimer et al. (Heimer et al., 2015) found that infection with *Giardia duodenalis* is a possible cause of stunting in children. The use of deworming tablets in children is a practice to be encouraged, especially in rural settings where children might be more prone to infections due to less hygienic environments and low levels of education on the part of caregivers (UN, 1993). The BMI of caregivers was a predictor of HAZ in our study population, which links to previous observations that mothers with a low BMI tend to have smaller babies (Branca & Ferrari, 2002).

Adequate nutrition during the preconception stage for future mothers is vital and could prevent intra-uterine growth retardation (WHO, 2012). Dietary zinc intake positively predicted HAZ, after taking into account the interaction term between energy and age. Although both variables were significant, their significance was not robust since they were not found to be significant in the sensitivity analysis nor were they predictors for stunting. Thus, we cannot conclude on the significance of the interaction terms.

Most children were being breastfed; only a half had been exclusively breastfed during the first 6 mo. Continued breastfeeding is a common practice in developing countries. Alvarado et al. (2005) and Roche et al. (2016) reported similar levels of continued breastfeeding in Afro-Colombian children of 15 mo and Ecuadorian children of 12-16 mo of age. Exclusive breastfeeding until 6 mo is not practised at the same level as continued breastfeeding. In our study, caregivers acknowledged that they stopped exclusively breastfeeding their children because the child wanted to eat, was sick or had colic. This shows that there is a need for a continued effort in educating caregivers about the importance and benefits of exclusive breastfeeding during the first 6 mo.

The number of children who had received vitamin A doses in the previous 6 mo was high (93%) whereas a small percentage (38%) of caregivers had used micronutrient powders (MNPs) in the previous 4 wk. MNPs are known to improve micronutrient status in children (Salam et al., 2013), but low compliance has been identified as a challenge in using them (Kristina Michaux et al., 2014).

Although Musanze district is a highly fertile region and is considered to be the food basket of Rwanda, we found out that for most children their diet was not diversified. There was no apparent difference between stunted and non-stunted children regarding food group intake, probably

because most of the children were having a non-diversified diet. This could be explained by the low wealth status of the participants and the higher price of animal source foods. However, a lack of knowledge on the part of caregivers about providing a balanced diet for children is also likely to play a role (Yue et al., 2016).

Nutrient intake from complementary foods was compared to the nutrient intake requirements for children. Overall, the nutrient intake of children was below the recommended levels. Considering absorbable zinc, dietary zinc intake was deficient across age groups because the children's diet was mostly plant-based. Not only was the diet poor in zinc but also there was poor availability due to the high phytates content of the diet. Flesh foods were consumed mostly in the form of small dried fish known as *indagara*. Dietary diversification focusing on increasing the consumption of locally available nutrient-rich foods, such as the small fish, could help in increasing children's intake of zinc.

Study strength and limitations

This study was conducted as a first necessary step in the process of scaling up on a national level the research on stunting in Rwanda. The strength of our study lies in the use of a multi-pass interviewing technique to minimize the recall bias and ensure correctness of the data collected. For the interpretation of the findings however, some limitations should be considered. Firstly, the size of our sample was small and might not have allowed us to capture extensively the predictors of stunting in Musanze District. Secondly, due to the cross-sectional nature of this study, we cannot establish causal relationships. Thirdly, because a single 24-hr recall was used, usual intake at the individual level could not be estimated. However, for comparing mean group level dietary intake, a single recall is acceptable (Gibson & Ferguson, 2008). Lastly, calculations for the nutrient content of foods

relied mainly on the use of yield, density and nutrient retention factors from published sources.

2.5 Conclusion

Our study shows the multifactorial nature of the stunting problem in the Northern Province of Rwanda. Age, exclusive breastfeeding and deworming tablet use in the previous 6 mo were all predictors of stunting in children with age > 12 mo of age, exposing children to stunting; whereas exclusive breastfeeding and use of deworming tablets were protective. Although not robust, the predictive effect of BMI of caregivers, dietary zinc intake and the interaction terms between age groups and energy intake on height-for-age z-scores was observed. Although most of the children were still breastfed, their complementary diet was often poor in essential nutrients for growth and development due to a predominantly plant-based diet. Public health messages focusing on the importance of the optimal nutritional status of women during the preconception period and exclusive breastfeeding within the first 6 mo need to be reinforced and sustained. Also, the use of deworming tablets needs to be encouraged, as it can contribute to reducing the burden that infections impose on a child's growth. A dietary diversification strategy of including locally available and affordable animal source foods in the diet of children is recommended.

Appendix 1. Appendix to Chapter 2. Data on child complementary feeding practices, nutrient intake and stunting in Musanze District, Rwanda[5]

[5] This appendix is based on a published data paper: Uwiringiyimana, V., Ocke, M. C., Amer, S., & Veldkamp, A. (2018). Data on child complementary feeding practices, nutrient intake and stunting in Musanze District, Rwanda. Data Brief, 21, 334-342. doi:10.1016/j.dib.2018.09.084

Abstract

Stunting prevalence in Rwanda is still a major public health issue, and data on stunting is needed to plan relevant interventions. This data, collected in 2015, presents complementary feeding practices, nutrient intake and its association with stunting in infants and young children in Musanze District in Rwanda. A household and a 24-hour recall questionnaire were used to collect the data. In total 145 children aged 5-30 months participated in the study together with their caregivers. The anthropometric status of children was calculated using Anthro software (WHO, 2011) according to the WHO growth standards (WHO, 2006b). The complementary feeding practices together with households' characteristics are reported per child stunting status. The nutrient intake and food group consumption are presented per age group of children. Also, the percentage contribution of each food groups to energy and nutrient intake in children was calculated. The data also shows the association between zinc intake and age groups of children. Using multiple linear regression, a sensitivity analysis was done with height-for-age z-score as the dependent variable and exclusive breastfeeding, deworming table use, BMI of caregiver, dietary zinc intake as independent variables. The original linear regression model and a detailed methodology and analyses conducted are presented in Uwiringiyimana et al. (Uwiringiyimana et al., 2018b).

Keywords: complementary feeding practices, stunting, nutrient intake, children, Musanze, Rwanda

Specifications Table

Subject area	Nutrition
More specific subject area	Nutritional status and complementary feeding practices
Type of data	Table, figure
How data was acquired	Household questionnaire, 24-hour recall questionnaire and anthropometric measurement
Data format	Analysed
Experimental factors	
Experimental features	
Data source location	Musanze District, Rwanda
Data accessibility	Data is with this article

Value of the data

The data is important for any program or intervention designed to alleviate stunting in children in Rwanda

- This data is useful to researchers looking for locally conducted research on stunting in children in Rwanda.
- This data is important for complementary feeding practices and stunting in children.
- The food group consumption data can be used for further research on the dietary intake of infants and young children.
- Programs or interventions aiming at improving the diet quality of children focusing on specific nutrients such as micronutrients can use our data as a benchmark of the quality of complementary foods that children consume.
- Our data is useful to inform government, local and international partners working to alleviate stunting in the African region.

Data

The data presents the child complementary feeding practices, nutrient intake and stunting status of children in Musanze District. Table 6 presents the anthropometric status of children, and their height-for-age, weight-for-age and weight-for-height z-scores. It also includes the stunting, wasting and undernutrition status. Table 7 shows the comparison of stunting, wasting and undernutrition in the District of Musanze and the national prevalence of stunting, wasting and undernutrition reported in the 2015 Demographic and Health Survey. Table 8 shows the complementary feeding practices and household characteristics per stunting status. Table 9 and Table 10 portrays the per cent contribution of food groups to energy and nutrient intake; specifically, Table 10 includes the micronutrient powder among the food groups. Table 11 shows the consumption of food groups per age groups in the same children population. Table 12 displays the association between dietary zinc intake and age groups of children using Kruskal-Wallis Test and Jonchheere-Terpstra Test. Figure 5, 6 and 7 are derived from table 12 and display the independent samples test view and pairwise comparisons. Lastly, table 13 is about the sensitivity analysis model conducted by considering children whose caregivers indicated that the food the child ate the previous day was similar to the child's usual intake.

Experimental Design, Materials and Methods

The data presented was obtained through a cross-sectional survey conducted in the district of Musanze. A detailed methodology is given elsewhere (Uwiringiyimana et al., 2018b). Ethical approval to collect the data was obtained through the Institutional Review Board of the College of Medicine and Health Sciences in Rwanda. An informed consent was obtained from all participating caregivers. A household questionnaire was used to collect information on socioeconomic status, complementary feeding practices, health and anthropometric status of

children. An interactive and multi-pass 24-hour recall questionnaire, adapted and validated for use in developing countries (Gibson & Ferguson, 2008), was used to collect information on dietary intake. A total of 145 children participated in the study. A single 24-hour recall with the caregiver as the respondent was conducted. Information on usual intake of children was also collected. Anthropometric status of children and their caregivers were collected and analysed using Anthro software (WHO, 2011). Extreme values for height-for-age z-scores were flagged and were considered as outliers during data analysis. Data processing of nutrient intake was done in Excel 2010. Statistical analysis was conducted using SPSS statistical software package version 24.

Table 6. Nutritional status of children between 5 to 30 months (n=138) in Musanze District, Rwanda

Characteristic	Mean ± SD	Frequency (N)	Percentage (%)
Anthropometric status			
Height-for-age z-score (HAZ)	-1.58 ± 1.77		
Weight-for-height z-score (WHZ)	0.22 ±1.32		
Weight-for-age z-score (WAZ)	-0.86 ± 1.31		
Stunting (HAZ <-2)*			44
Moderate stunting		38	62
Severe stunting		23	38
Wasting (WHZ <-2)*			7
Moderate wasting		6	61
Severe wasting		4	39
Underweight (WAZ <-2)*			16
Moderate underweight		18	78
Severe underweight		5	22

*The percentage (%) for moderate and severe categories are given within the respective group of stunting, wasting and underweight.

Table 7. Anthropometric status of children aged 5-30months (n=138) in Musanze District compared to national prevalence of under 5

Indicator	Prevalence (Musanze)	National prevalence[1]
Stunting	44	38
Underweight	16	9
Wasting	7	2

[1] Rwanda Demographic and Health Survey 2015-16 (NISR et al., 2015)

Table 8. Complementary feeding practices and household characteristics of children between 5 to 30 months in Musanze District, Rwanda

	Non-stunted (n=77)	Stunted (n=61)	Total (n=138)	
Characteristic		N (%)		p-value*
Complementary feeding practices				
Pre-weaning food				
Plain water	2 (7)	10 (24)	12 (18)	-
Cow milk	2 (8)	2 (5)	4 (6)	
Traditional herbal mixture	7 (27)	13 (31)	20 (29)	
Fruit juice	6 (23)	10 (24)	16 (24)	
Porridge	7 (27)	4 (9)	11 (16)	
Other	2 (8)	3 (7)	5 (7)	
Reason for pre-weaning				
Inadequate breast milk	3 (12)	3 (7)	6 (9)	-
Sickness of child	7 (27)	11 (26)	18 (26)	
Colic disease	4 (15)	8 (19)	12 (18)	
Child wanted to eat	10 (38)	13 (31)	23 (34)	
Other	2 (8)	7 (17)	9 (13)	
Weaning age groups				-
<6 months	0 (0)	1 (25)	1 (9)	
7-12 months	3 (43)	1 (25)	4 (36)	
13-24 months	4 (57)	2 (50)	6 (55)	
Person responsible for feeding the child				0.022[a]
Respondent	75 (99)	54 (86)	129 (94)	
Other	1 (1)	7 (12)	8 (6)	
Usual food consumed				
Yes	61 (81)	57 (93)	118 (87)	0.038
No	14 (19)	4 (7)	18 (13)	
Household characteristics				

Ownership of agricultural land				0.644
Self-owned	31 (56)	27 (61)	58 (59)	
Hired	17 (31)	10 (23)	27 (27)	
Self-owned & hired	7 (13)	7 (16)	14 (14)	
Income generating activity				0.690
None	5 (7)	6 (10)	11 (8)	
Commerce	8 (10)	6 (10)	14 (10)	
Agriculture	40 (52)	25 (41)	65 (48)	
Domestic work	18 (24)	19 (31)	37 (27)	
Employment (formal &informal)	5 (7)	5 (8)	10 (7)	
Water source for household				-
Piped water	58 (76)	43 (70)	101 (73)	
Water from spring	4 (5)	7 (12)	11 (8)	
Rainwater	2 (3)	3 (5)	5 (4)	
Surface water (river /dam/ stream)	12 (16)	8 (13)	20 (15)	
Water treatment in the household				
Nothing	38 (51)	34 (56)	72 (53)	-
Boil	26 (35)	19 (31)	45 (33)	
Add bleach/chlorine	7 (9)	6 (10)	13 (10)	
Other	4 (5)	2 (3)	6 (4)	
Time taken to/from water collection point				0.181
Less than 30 min	49 (64)	32 (53)	81 (59)	
Between 30-60 min	19 (25)	16 (26)	35 (26)	
More than 1 hour	8 (11)	13 (21)	21 (15)	
Biofortified crops grown by household				0.445 [a]
Yes	0 (0)	1 (2)	1 (1)	
No	76 (100)	60 (98)	136 (99)	
Improved seeds use by household				0.754 [a]
Yes	7 (9)	4 (7)	11 (8)	
No	69 (91)	57 (93)	126 (92)	
Industrial fertilizers use by household				0.801
Yes	47 (62)	39 (64)	86 (63)	
No	29 (38)	22 (36)	51 (37)	

*p-value: two-sided, obtained through Pearson Chi-square. [a] Exact Sig. (2-sided) from Fisher's Exact Test. - If n was too low for statistical testing.

Table 9. Percent contribution of food groups to energy and nutrient intake from complementary feeding of children (aged 5-30 months) from Musanze District[1]

Food groups	Energy	Protein	Fat	Carbohydrate	Iron	Calcium	Magnesium	Zinc	Phytates	Vitamin A	Vitamin C
Cereals	35	45	13	58	22	14	49	32	52	0	0
Roots and tubers	4	3	1	8	2	2	4	3	4	0	9
Legumes	3	10	0	5	6	6	8	7	20	2	4
Nuts, seeds and their products	5	10	6	2	3	1	10	6	22	0	0
Milk and milk products	1	1	0	1	0	3	1	1	0	0	0
Meat, poultry, fish	3	15	3	0	3	23	5	17	0	0	0
Egg or egg products	1	5	1	0	1	1	0	2	0	1	0
Fruits and fruit juices	4	2	1	7	2	1	6	2	0	1	22
Vegetables, herbs and vegetable products	5	10	1	8	60	48	18	15	1	18	64
Fats and oils	36	0	72	0	0	0	0	15	0	77	0
Sugar and sweets	5	0	0	12	1	0	0	0	0	0	0

[1] Micronutrient powder (MNP) was not included

Table 10. Percentage contribution of food groups to energy and nutrient intake from complementary feeding with micronutrient powder (MNP) included[1]

Food groups	Energy	Protein	Fat	Carbohydrate	Iron	Calcium	Magnesium	Zinc	Phytates	Vitamin A	Vitamin C
Cereals	34	45	13	55	5	14	49	6	52	0	0
Roots and tubers	4	3	1	7	0	2	4	0	4	0	2
Legumes	3	10	0	5	1	6	8	1	20	1	1
Nuts, seeds and their products	5	10	6	1	1	1	10	1	22	0	0
Milk and milk products	1	1	0	1	0	3	1	0	0	0	0
Meat, poultry, fish	3	15	3	0	1	23	5	3	0	0	0
Egg or egg products	1	5	1	0	0	1	0	0	0	1	0
Fruits and fruit juices	4	2	1	6	0	1	6	0	0	0	6
Vegetables, herbs and vegetable products	5	10	1	8	12	48	18	3	1	7	17
Fats and oils	36	0	72	0	0	0	0	3	0	28	0
Sugar and sweets	5	0	0	11	0	0	0	0	0	0	0
Other (MNP)	0	0	0	4	80	0	0	82	0	63	74

[1]Micronutrient powder had been used by only 38% of caregivers in the last four weeks that preceded the survey. No caregiver had used micronutrient powder in their child's diet the day that preceded the survey.

Table 11. Prevalence of food group consumption reported in a single 24-hour recall in children aged 5-30 months from Musanze District

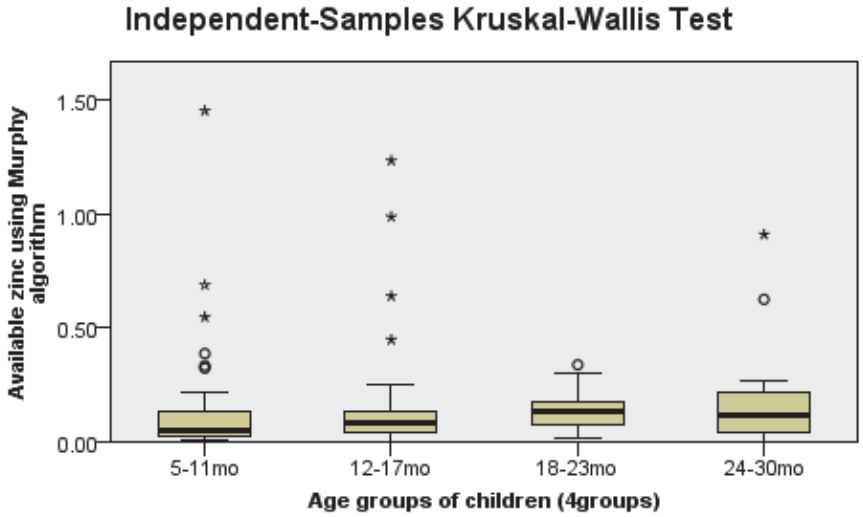
		5-11mo (n=49)	12-17mo (n=46)	18-23mo (n=35)	24-30mo (n=14)	Total (n=144)
Food groups				N (%)		
Grain, roots & tubers	No	1 (1)	3 (2)	0 (0)	1 (1)	5 (3)
	Yes	48 (33)	43 (30)	35 (24)	13 (9)	139 (97)
Legumes & nuts	No	8 (6)	11 (8)	8 (6)	4(3)	31 (22)
	Yes	41 (28)	35 (24)	27 (19)	10 (7)	113 (78)
Dairy products (milk, yogurt, cheese)	No	46 (32)	46 (32)	35 (24)	14 (10)	141 (98)
	Yes	3 (2)	0 (0)	0 (0)	0 (0)	3 (2)
Flesh foods (meat, fish, poultry & liver/organ meats)	No	44 (31)	41 (28)	35 (24)	13 (9)	133 (92)
	Yes	5 (3)	5 (3)	0 (0)	1 (1)	11 (8)
Eggs	No	49 (34)	46 (32)	32 (22)	14 (10)	141 (98)
	Yes	0 (0)	0 (0)	3 (2)	0 (0)	3 (2)
Vitamin A rich fruits & vegetables	No	11 (8)	11 (8)	9 (6)	5 (3)	36 (25)
	Yes	38 (26)	35 (24)	26 (18)	9 (6)	108 (75)
Other fruits & vegetables	No	22 (15)	24 (17)	23 (16)	10 (7)	79 (55)
	Yes	27 (19)	22 (15)	12 (8)	4 (3)	65 (45)

Table 12. Association between zinc intake and age groups (Kruskal-Wallis test)

Hypothesis Test Summary				
	Null Hypothesis	**Test**	**Sig.**	**Decision**
1	The distribution of Available zinc using Murphy algorithm is the same across categories of Age groups.	Independent-Samples Kruskal-Wallis Test	.028	Reject the null hypothesis.
2	The distribution of Available zinc using Murphy algorithm is the same across categories of Age groups.	Independent-Samples Jonckheere-Terpstra Test for Ordered Alternatives	.005	Reject the null hypothesis.
Asymptotic significances are displayed. The significance level is .05.				

There was a statistically significant difference in zinc intake between age groups, H (3) = 9.12, p = 0.028. Pairwise comparisons with adjusted p-values showed that there was a significant difference in zinc intake between the age group of 5-11 months and 18-23 months (p = 0.021). On the other hand, there was no significant difference in zinc intake between age group of 5-11 months compared to the age group 12-17 months (p = 1.00) and 24-30 months (p = 1.00). There were also no significant differences in zinc intake between the age group of 12-17 months and the age groups of 24-30 months (p = 1.00) and age group of 18-23 months (p = 0.195). Finally, there were no significant differences in zinc intake between the age groups of 24-30 months and the age group of 18-23 months (p = 1.00).

The Jonchheere-Terpstra's test revealed a significant trend in the data: as the age of children increased, zinc intake increased, J = 4471, z = 2.794, p = 0.005.

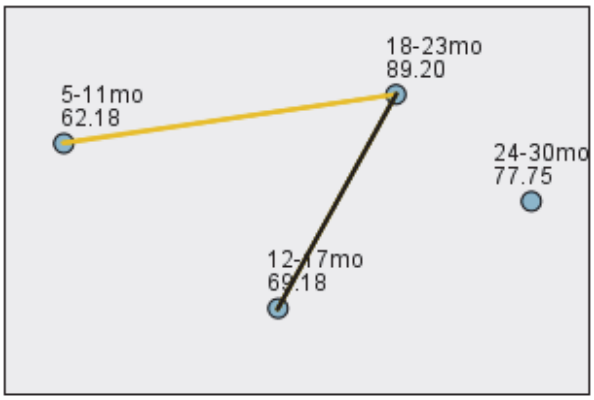


Total N	144
Test Statistic	9.119
Degrees of Freedom	3
Asymptotic Sig. (2-sided test)	.028

1. The test statistic is adjusted for ties.

Figure 5. Association between zinc intake and age groups: Independent samples test view for Kruskal-Wallis Test

Pairwise Comparisons of Age groups of children (4groups)

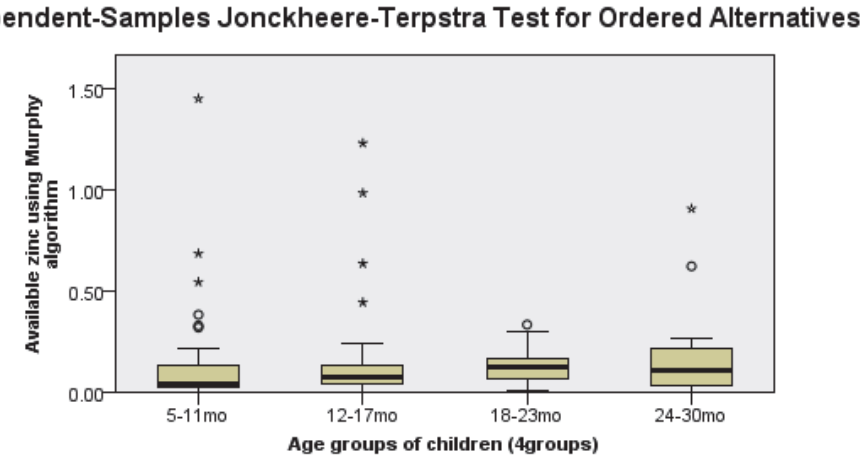


Each node shows the sample average rank of Age groups of children (4groups).

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
5-11mo-12-17mo	-7.001	8.564	-.818	.414	1.000
5-11mo-24-30mo	-15.566	12.641	-1.231	.218	1.000
5-11mo-18-23mo	-27.016	9.232	-2.926	.003	.021
12-17mo-24-30mo	-8.565	12.732	-.673	.501	1.000
12-17mo-18-23mo	-20.015	9.356	-2.139	.032	.195
24-30mo-18-23mo	11.450	13.191	.868	.385	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.
Significance values have been adjusted by the Bonferroni correction for multiple tests.

Figure 6. Association between zinc intake and age groups: Pairwise comparisons for Kruskal-Wallis Test



Total N	144
Test Statistic	4,471.500
Standard Error	276.263
Standardized Test Statistic	2.794
Asymptotic Sig. (2-sided test)	.005

Figure 7. Association between zinc intake and age groups: Independent samples test view for Jonchheere's Test for Ordered Alternatives

Table 13. Sensitivity analysis model of predictors of height-for-age z-scores in children aged 5-30 months in Musanze District, Rwanda[1]

Variables	β	*p*-value	95% CI for β	
			Lower bound	Upper bound
Age (months)				
Age group 12-17mo vs 5-11mo	-0.92	0.000	-7.55	-3.10
Age group 18-23mo vs 5-11mo	-2.19	0.073	-1.94	0.09
Age group 24-30mo vs 5-11mo	-2.83	0.000	-3.13	-1.25
Exclusive breastfeeding (yes)	0.79	0.001	-4.43	-1.23
Use of deworming tablets (yes)	1.93	0.005	0.23	1.35
BMI of caregiver (kg/m2)	0.12	0.006	0.03	0.21
Dietary zinc intake (mg)	1.13	0.178	-0.52	2.79
Interaction terms between age groups and energy intake				
Age group 12-17mo*energy intake	-0.002	0.080	-0.004	0.000
Age group 24-30mo*energy intake	-0.002	0.175	-0.005	0.001

[1]The sensitivity analysis model was limited to 116 children whose intake on the recalled day was similar to their usual intake. β: Regression coefficient. CI, confidence interval.

Chapter 3 Exposure to aflatoxins from maize and peanut flours and stunting in young children from the Northern region of Rwanda[6]

[6] This chapter is based on Uwiringiyimana, V., Ocke, M. C., Amer, S., & Veldkamp, A. Exposure to aflatoxins from maize and peanut flours and stunting in young children from the Northern region of Rwanda (To be submitted to the Food and Nutrition Bulletin).

Abstract

Maize and peanuts are known to be highly susceptible to aflatoxins contamination. And because they are staple foods in Rwanda, their consumption through complementary meals poses a constant risk of exposure to young children. We analysed the total aflatoxins levels in maize and peanut flours, estimated the exposure to aflatoxins in young children and studied the relationship between stunting and exposure to aflatoxins. A cross-sectional survey of 145 children aged 5-30 months was conducted in Musanze District, Rwanda. The dietary intake of maize and peanut flour was collected using a 24-hour recall questionnaire. A total of 71 samples of maize and peanuts were collected from households, centres and the main market in the region. The aflatoxins content was analysed using a single-step lateral flow assay (Reveal Q+). The dietary exposure to aflatoxins was derived from the maize and peanut consumption data and the median aflatoxins content in maize and peanut flours. Stunted children consumed significantly higher amount of maize flour than their non-stunted counterparts (p-value < 0.05). The mean aflatoxins content of maize and peanut from households was 3.02 μg/kg and 181.40 μg/kg respectively. The level of aflatoxins in all the peanut samples exceeded regulatory limit for aflatoxins in East-Africa (10 μg/kg). The total aflatoxins exposure was negatively associated with the height-for-age (p-value < 0.05) after adjusting for the age of a child, breastfeeding status, use of deworming tablets, body mass index of caregiver and dietary zinc intake. Our findings indicate that children in Musanze are exposed to aflatoxins through the consumption of contaminated maize and peanut flours which appear also to be contributing to the stunting levels observed in children.

Keywords: *aflatoxins, maize, peanuts, complementary feeding, stunting, Rwanda*

3.1 Introduction

Mycotoxins are toxic secondary metabolites produced by moulds that contaminate various agricultural commodities either before harvest in the fields or under post-harvest conditions. Mycotoxins are known to be carcinogenic, mutagenic, teratogenic and immunosuppressive (FAO, 2001). Among them, the most abundant and more toxic to humans are aflatoxins. These are proven human carcinogens as they are directly associated with the occurrence of liver cancer (Liu & Wu, 2010; Udomkun et al., 2017). Aflatoxins are produced by *Aspergillus flavus*, *A. parasiticus* and *A. nominus*, and are commonly associated with food commodities during drying and storage *(Pitt, 2000)*. The *Aspergillus* occur naturally in nature and can easily infect cereals and nuts in places where environmental conditions are favourable such as high temperatures, moisture, unseasonal rain and floods (Bhat & Vasanthi, 2003). Besides, poor harvesting practices, improper storage and less than optimal conditions during transport can contribute to fungal growth and aflatoxins production (WHO, 2003).

In Africa, the largest recorded outbreak of aflatoxicosis occurred in Kenya in 2004 with 317 cases and 125 recorded deaths (CDC, 2004). Concentrations of aflatoxin B_1 in maize were found to be as high as 8000 ppb which was 400 times greater than the 20 ppb, acceptable limit for food (CDC, 2004). Acute aflatoxicosis can be characterized by haemorrhage, acute liver damage, oedema and death (Khlangwiset et al., 2011). Though the episodes of acute aflatoxicosis are not recurrent, chronic human exposure to aflatoxins occurs through daily consumption of maize and peanuts that compose most of the diet of the population in Sub-Saharan Africa (WHO, 2006a; Udomkun et al., 2017). High levels of aflatoxins were reported in Sub-Saharan countries such as in maize from Malawi (Mwalwayo & Thole, 2016), in peanuts from markets in Kenya (Mutegi et al., 2013) in milk and animal feeds from Ethiopia (Gizachew et al., 2016), in maize-based complementary foods from Tanzania

(Kamala et al., 2017) and in maize and peanuts from Burundi and Eastern Democratic Republic of Congo (Udomkun et al., 2018). Maize and peanuts alone dominate in contributing to the high exposure to aflatoxins in comparison to other food commodities (Amuzie et al., 2016).

Although there is increasing recognition and awareness of the health and economic impact of aflatoxins, in many developing countries enforcement of the regulatory measures for aflatoxins focuses more on the food stuffs destined for the international trade (Wild & Gong, 2010). While there is growing momentum in various Sub-Saharan countries on the reduction of aflatoxins in foods, a lack of capacity to enforce the monitoring of aflatoxins levels in the locally consumed maize and peanuts continue to pose a risk of chronic exposure to the local population (Shephard, 2008b). Chronic mycotoxins exposure in children is particularly detrimental to their wellbeing as evidence shows that it results into linear growth retardation or stunting (Gong et al., 2004; Khlangwiset et al., 2011). This chronic exposure was shown by the detection of aflatoxins and aflatoxin-albumin adducts in utero, in the cord blood of babies, maternal breast milk, in aflatoxin-albumin adducts of young children (Gong et al., 2004; Egal et al., 2005), and in weaning diets (Gong et al., 2003; Owaga et al., 2011). It was also found that the exposure to aflatoxins markedly increases following the weaning period of children; emphasizing the critical importance of the quality of complementary foods on the linear growth in children (Gong et al., 2003; Gong et al., 2004; Khlangwiset et al., 2011). Stunting or growth retardation is defined as a height-for-age that is less than -2SD of the z-score cut-off point of the WHO reference population (de Onis et al., 2006). Stunting develops within the first 1000 days of life of a child and once established it is hardly reversible (Martorell et al., 1994). Stunting also leads to short adult height, increased risk of chronic diseases, lower attained schooling due to delay in cognitive functions development

which later leads to reduced adult income and productivity (Victora et al., 2008; Dewey & Begum, 2011). The majority of stunted children live in Asia and Africa. During the last decade, rates of stunting are in decline worldwide but remain high in Africa; currently, with 36.7% of children less than five years stunted, the East-Africa region is among the areas with the highest stunting levels (FAO et al., 2017). In Rwanda, 38 % of all children under five years were found to be stunted (NISR et al., 2015) which, according to WHO cut-offs, is classified as a very high prevalence (de Onis et al., 2019).

In Rwanda, maize and peanuts are an integral part of the diet. They are incorporated into complementary dishes for children as a source of energy and nutrients. Additionally, most maize and peanuts consumed in Rwanda are locally produced. However, because of the high market demand, they are also imported from neighbouring countries such as Tanzania or Uganda. A few studies have been conducted to identify the levels of aflatoxins contamination in maize and peanuts sold on the Rwandan market (Nyinawabali et al., 2013; Nishimwe et al., 2017; Umereweneza et al., 2018). However, no study has yet been conducted to analyse the aflatoxins contamination in complementary flours collected from households, and also assess the effect of aflatoxins exposure on the linear growth of children in Rwanda. Thus, this study aimed to analyse the level of aflatoxins contamination in maize and peanut flours used as complementary flours, to estimate the exposure to aflatoxins in young children in the Northern region of Rwanda, and finally assess the influence of the exposure to aflatoxins on stunting observed in children.

3.2 Methods

Study area and population

The survey was conducted in the District of Musanze located in the Northern region of Rwanda. Musanze District is characterized by a

mountainous topography, slopes and frequent rainfall. It is a highly fertile region due to volcanic soils. However, it is among the districts with high stunting levels (NISR et al., 2015). The district was chosen based on the outcome of a previous study that studied the determinants of stunting with particular focus on complementary feeding practices (Uwiringiyimana et al., 2018b). The survey took place in May 2015 and included 145 children from randomly selected households located in Gataraga and Busogo sectors of the District of Musanze. Figure 8 shows the geographical location of the households in the survey. Details of sampling and recruitment of the study population and data collection have been described before (Uwiringiyimana et al., 2018b).

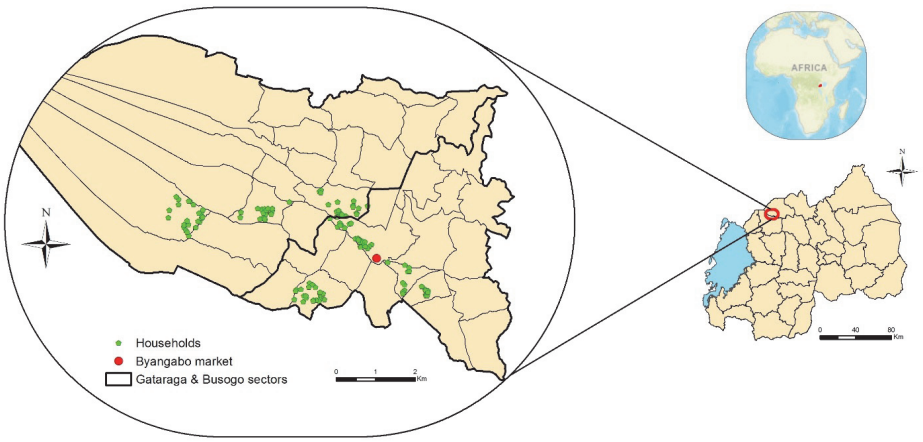


Figure 8. Geographical location of surveyed households in Northern Rwanda

In summary, the households participating in the survey were selected using a cluster-random sampling using villages in Musanze District as the primary sampling unit and households as the basic sampling units. All households with a child between 5-30 months had an equal chance to participate in the study. None of the households selected refused to participate in the study. The ethical approval to conduct the survey was obtained from the Institutional Review Board of the College of Medicine and Health Sciences of the University of Rwanda. Every survey participant signed an informed consent form translated in the local

language after the research objectives were explained to them. The socio-demographic characteristics of the caregivers, breastfeeding and complementary feeding practices were also collected as part of the survey.

Complementary food intake survey

The maize and peanut intake of children were obtained through a complementary food intake survey conducted using a one day 24-hour recall questionnaire adapted and validated for use in developing countries (Gibson & Ferguson, 2008). As in most cases, maize or peanut flour was consumed as part of a recipe such as porridge; the recipe information was asked for in terms of ingredients and their amounts, and as well as the quantity consumed by the child. The frequency of maize and peanut consumption per child was also collected as part of the survey. From the dietary data collected on the 24-hour recall questionnaire, the nutrient intake and the amount of maize and peanut consumed by each child were estimated using Microsoft Excel 2010 following the guidelines by FAO (Greenfield & Southgate, 2003). The recipe information collected was used to estimate the individual intake of flours per child. The computation of maize and peanut intake was calculated in grams after taking into account the ingredients quantities in the recipe, the edible portions (USDHEW & FAO, 1968; Rand et al., 1991; Vasco et al., 2008) to account for inedible parts, the yield factors (Bergström, 1994; Bognár, 2002; Stadlmayr et al., 2012) to adjust from raw to cooked weights, the volume of the porridge or food consumed by the child, and the density factors (FAO, 2012) to convert the consumed volume into grams equivalent. The daily maize consumption was estimated in g/kg body weight (bw)/day by dividing the maize consumption (in g) with the weight (in kg) of every child. The same procedure was applied for the estimation of daily peanut consumption. For reporting, the daily maize and peanut consumption was expressed in mg/kg bw.

Anthropometric measurements

As part of the survey, anthropometric measurements of children were taken: recumbent length of every child was measured using a measuring board with a fixed headrest and a movable food piece, designed by UNICEF. The length of every child was recorded to the nearest 0.1 cm. The weight of children was measured to the nearest 0.1 kg using an electronic scale (WHO, 1995). Height-for-age z-scores (HAZ) were calculated using the Anthro software by the World Health Organization (WHO) where z-scores < -2 indicate stunting (WHO, 2011).

Sampling of maize and peanut flour

The households participating in the dietary survey were asked to provide a sample of the maize and peanut flour used to cook the food consumed by the child in the household. To not put a strain on the household, a limited quantity between 200g and 500g was collected depending on what was available in the household. The samples were placed in a plastic bag and sealed and were kept under cold storage until analysis. A total number of 45 samples (Table 14) were collected in 40 households out of the 145 households visited, because not every household had maize or peanut flour at the time of the visit. Maize grains were requested in case the household did not have maize flour. In addition, maize and peanut flour samples were collected from local centres in the neighbourhoods where there are shops from which the local population usually get their supply for maize and peanut flour. Samples were also collected from the main market, Byangabo market, which supplies the local shops in the neighbourhoods.

Table 14. Type of samples collected in 2015 in Musanze District per sample source and per batch of 2015 and of 2016.

Sample source	Sample type	N (batch of 2015)	N (batch of 2016)
Households	Maize flour	10	
	Maize grains	25	
	Peanut flour	10	
Centers	Maize flour	3	
	Maize grains	1	
	Peanut flour	3	3
Markets	Maize flour	3	
	Maize grains	6	
	Peanut flour	4	3

Similar to the households' selection, the sampling of the shops in the local centres and Byangabo market was randomly done. In total, seven samples were collected in the centres, and thirteen samples were collected in Byangabo market (Table 14). A second batch of six peanut flour samples was collected in 2016 at the Byangabo market, and from the local centres that were previously visited in 2015. Only peanut flour samples were collected in the second batch because, in the first batch, peanuts were more contaminated than maize flour samples.

Aflatoxins determination in ready-to-cook maize and peanuts

The total aflatoxins content of maize and peanut flours were determined using the Reveal Q+ for Aflatoxins testing (Neogen Corporation, Lansing, MI USA), approved by the USDA Grain Inspection, Packets and Stockyard Administration (GIPSA) (USDA & GIPSA, 2015). Reveal Q+ for Aflatoxin is a single-step lateral flow immunochromatographic assay based on a competitive immunoassay format. Lateral flow strips coated with aflatoxin antibodies react with the aflatoxin content in the sample extract. The analysis was conducted according to the manufacturer's directions and the calibration of the reader was

performed also as per protocol from the manufacturer. From each sample collected, 10 g was weighed after thoroughly mixing and transferred into a sample cup. Maize grains collected were first ground to obtain a fine flour. For extraction in maize samples, 50 ml of 65% ethanol was measured and transferred to the sample cup. The mixture was vigorously shaken for three min and left to settle. The supernatant was then filtered using Whatman No. 1 filter paper, and 100 µl of the extract was transferred to a dilution cup where 500 µl of sample diluent had been previously added. From the mixture, 100 µl was transferred to a sample cup where a Reveal Q+ Aflatoxin strip was promptly inserted and left to develop for six minutes. After, the developed strip was directly inserted into a Reveal AccuScan III Reader System (AS 4912, Neogen Corporation, Lansing, MI USA) and the reader displayed in parts per billion (ppb) the total aflatoxins content of the sample. For aflatoxins determination in peanuts, the same procedure was followed except for using 1 parts sample to 3 parts 65% ethanol for the extraction process. The limit of detection was 2 µg/kg, and the range of quantification was 2-150 ppb. The extraction process was repeated if the reading came as >150 ppb. Most peanut samples required one extra dilution to have a reading below 150 ppb. All samples were analysed in duplicates. For validation of the readings, two aflatoxin standard reference materials spiked with 4.5 µg/kg and 11.9 µg/kg were used to estimate the mean recovery and the coefficient of variation.

Estimation of exposure to aflatoxins

The exposure to aflatoxins from maize and peanut was estimated for each child in the survey using his/her maize and peanut consumption together with the median aflatoxins content of the maize and peanut flours collected in all the households. The dietary exposure to aflatoxins from maize for each child was determined using the following formula:

Dietary exposure to aflatoxin from maize (μg/kg bw/day) = Average daily maize consumption (g/kg bw/day) x median aflatoxin content (μg/kg)/1000 in the maize flour sample from all households.

A similar formula was applied in the case of calculating dietary exposure from peanut intake. After, the dietary aflatoxins exposure values from maize and peanut were summed up to come up with the total aflatoxins exposure. The median aflatoxins content of maize and peanut samples was used because not all households had maize or peanut flour at the time of the interview. Thus we couldn't estimate individual exposure to aflatoxins. The use of median or mean aflatoxin content of flour has been applied in other studies (Ortiz, 2014; Hove et al., 2016).

Statistical analysis

Descriptive statistics were conducted for all variables, and continuous variables were checked for normality. Median values were reported in case the variables were not normally distributed. Independent-samples Man-Whitney U test was used to compare the daily maize flour intake and peanut flour intake by stunting status. Kruskal-Wallis test was used to compare the dietary intake of maize and peanut flours per age groups of children. Linear regression analysis was conducted to study the association between height-for-age of children and the exposure to aflatoxins by adjusting for other variables known to influence stunting in the study population (Uwiringiyimana et al., 2018b). A p-value of <0.05 indicated statistical significance. Statistical analysis was performed using SPSS version 24.

3.3 Results and discussion

Maize and peanut consumption

Table 15 gives an overview of the study population. 145 children participated in the study, 52% of children were girls, and 48% were

boys. From Gatagara and Busogo sectors, 83 and 62 households were included, respectively. Among the caregivers, mothers (95%) of the children were the primary respondents.

Caregivers responded about their child intake of maize and peanut. Most caregivers (96%) reported that they feed their children maize, all (100%) in the form of porridge (Table 16). The porridge was cooked thin or thick depending on the age of the child. Sometimes mothers mix millet, wheat or soy flour with maize flour to make the porridge. For peanuts, 78% of mothers affirmed that they feed their children peanut flour which is always (100%) cooked as soup or incorporated into complementary dishes to make stew.

Table 15. Characteristics of the study population of 145 children aged 5-30 months in Musanze, Rwanda[†]

Characteristic	N (mean)	% (SD)
Sex of child		
Female	72	52
Weight of child (kg)	(8.9)	(1.6)
Respondent		
Mother	130	95
Caregiver education		
Illiterate	30	22
Primary education	92	67
Secondary to tertiary education	15	11
Caregiver age (years)	(28)	(8)
BMI of caregiver (kg/m^2)	(23.3)	(3.2)
Location of households		
Gatagara sector	83	57
Busogo sector	62	43

[†]Valid percentages (%) are represented

Figure 9 shows the consumption of maize and peanut per week by the study population. About half of all children (48%) consumed maize every day while peanuts were consumed less frequently, i.e. 13% of the

children consumed it four times or more per week and one third (33%) less than two times a week. The frequency of maize intake was higher because maize is a staple crop in the region, and is more available and affordable for the household.

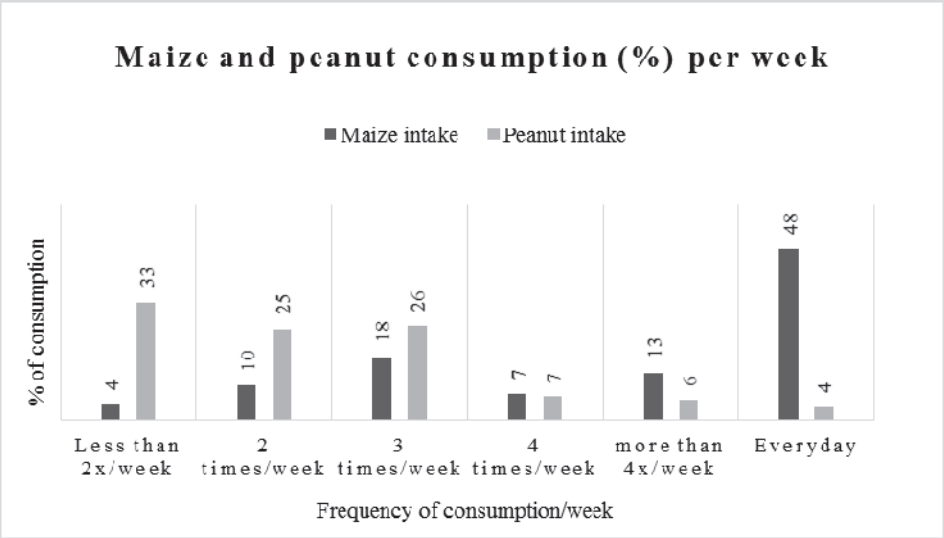


Figure 9. Consumption frequency of maize and peanuts (%) by children aged 5-30 months in Musanze District, Rwanda

Moreover, most of the maize consumed in the households was from the household farm (92%) while peanut flour was nearly always (99%) purchased from the market (Table 16).

Table 16. Complementary feeding practices, maize consumption and peanut consumption in the children aged 5-30 months in Musanze District, Rwanda[†]

Characteristic	N (mean)	% (SD)
Exclusive breastfeeding	69	50
Current breastfeeding	125	92
Age groups at the introduction of complementary foods		
1-5mo	38	28
6-9mo	96	72
Deworming tablets use in the last six months	99	73
Food group consumption during the recalled day		
Grains, roots & tubers	140	97
Legumes & nuts	114	79
Dairy products (milk, yoghurt, cheese)	3	2
Flesh foods (meat, fish, poultry & organ meats)	11	8
Vitamin A rich fruits & vegetables	109	75
Other fruits & vegetables	66	46
Maize consumption by the child		
Yes	137	96
Peanut consumption by the child		
Yes	112	78
Source of maize in the household		
Household farm	127	92
Purchased from market	11	8
Source of peanut in the household		
Household farm	1	1
Purchased from market	113	99
Storage duration (days)		
Maize flour	(16)	(32)
Peanut flour	(2)	(7)
Form in which maize is fed to children		
Maize flour as porridge	137	100
Form in which peanuts are fed to children		
Peanut flour (as soup or added to dishes)	112	100

[†]Valid percentages (%) are represented.

Peanut flour is usually more expensive than maize flour per kilo. Thus, even if mothers would have wanted to frequently cook peanut flour for their children, they could not afford to do so every day. More descriptive statistics on anthropometric status, complementary feeding

practices and household characteristics of children are presented elsewhere (Uwiringiyimana et al., 2018a).

Aflatoxin occurrence in maize and peanut

The mean recovery (%) and the coefficient of variation (%) were 113.3 (8.3) and 66.4 (10.7) for the reference material with 4.5 μg/kg and 11.9 μg/kg spike of total aflatoxins respectively. The aflatoxins content of maize and peanut flours are shown per households in table 17. All the samples from households tested positive for aflatoxins. The mean total aflatoxins content of samples collected from households was 3.0 μg/kg and 181.4 μg/kg for maize flour and peanut flour respectively. The range of contamination was 1.5-5.2 μg/kg for maize flour and 68.0-269.0 μg/kg for peanut flour. Peanut flour samples were more contaminated compared to maize flour samples.

Table 17. Total aflatoxins content (μg/kg) in maize and peanut from households in the District of Musanze

	Total aflatoxins contamination (μg/kg)	
	Maize flour (n=35)	Peanut flour (n=10)
Mean ± SD	3.0 ± 0.9	181.4 ± 55.4
Median	2.8	193.9
Range	1.5 – 5.2	68.0 – 269.0
25^{th}-75^{th}	2.4-3.4	147.8-217.3

SD: standard deviation

Table 18 represents the total aflatoxins content of maize and peanut flour samples collected from all sample sources: households, centres and Byangabo market. Table 5 also presents the percentage of positive samples according to three regulatory limits. 6% of maize flour and 9% of maize grains were found to be higher than the regulatory limit of aflatoxins in foods of 4 μg/kg in the European Union (EU), and none of the maize flour samples exceeded the regulatory limit of 10 μg/kg in the East-African Community (EAC) and 20 μg/kg in the USA (Table 18).

Table 18. Total aflatoxins content of maize flour, maize grains and peanut flour collected from households, centers and the Byangabo market in Musanze District, Rwanda

		Aflatoxins (μg/kg)		Positive samples (%)		
	n	Mean ±SD	Range	>4 μg/kg	>10 μg/kg	>20 μg/kg
Maize flour	16	2.9±0.8	1.5-5.2	6	0	0
Maize grains	32	2.9±0.9	2.1-5.2	9	0	0
Peanut flour	17	192.5±54.0	68.2-269.0	100	100	100
Peanut flour[†]	6	222.9±165.2	123.5-541.2	100	100	100

Maximum tolerable limit for aflatoxins in the EU (4 μg/kg), the EAC (10 μg/kg) and the USA (20 μg/kg). [†]Collected in the second batch of 2016

Although the maize samples were not contaminated beyond the regulatory limits, the low levels do not necessarily mean that the maize flour is safe. This is because our method reported only total aflatoxins levels and not the individual aflatoxins such B1, B2, G1 and G2. The regulatory limit for aflatoxins B1, known as the most potent aflatoxin, is 5 μg/kg for EAC and 2 μg/kg for EU (FAO, 2004). On the other hand aflatoxins levels from all peanut flour samples were higher than the regulatory limits accepted in the EU, in the EAC and the USA (FAO, 2004). In the Western Province of Rwanda, Nyinawabali et al. (2013) found levels of total aflatoxins as high as 3000 μg/kg in maize flour and as high as 1700 μg/kg in peanut flour. The levels of aflatoxins can significantly vary depending on the sample collected, the environmental and storage conditions. In our study, these factors might explain the low levels of aflatoxins found in maize. On the other hand, the fact that peanut flour is not grown locally and is transported over a long road distance of around 100 km from Kigali to Musanze could explain the high levels of aflatoxins found in peanuts. It is known that moulds can grow exponentially during storage in condition of heat and high humidity (Villers, 2014). Thus, the risk of increased fungi growth and proliferation, and aflatoxin production during transport could be

playing a role. In the Democratic Republic of Congo, Kamika et al. (2016) found that 32% of the samples that were contaminated in the pre-harvest period, along the supply chain the contamination level increased to 100% with all the samples becoming highly contaminated. The risk of fungi growth rises also depending on the packaging material used for storing and transportation of peanuts. In Rwanda, peanuts kernels are usually packaged and stored in propylene bags, and transported in open air on trucks. In Kenya, Mutegi et al. (2013) found that samples stored in plastic, propylene, PVC bags had significantly higher levels of aflatoxins compared to sample stored in jute bags. One of the solutions to reduce aflatoxins production during storage is the use of hermetic storage during post-harvest storage and transport of crops susceptible to aflatoxins production (Villers, 2014). Navarro et al. (2012) showed that hermetic storage significantly inhibited mould growth during storage of peanuts.

Figure 10 presents the aflatoxins contamination in maize (panel a) and peanut (panel b) by sample source. The contamination level was similar among the surveyed households, centres and market. The fact that the samples were collected in the same geographical area with similar environmental conditions might explain the comparable values in aflatoxins levels. Moreover, most of the peanut flour purchased by the households is directly consumed; this observation was shown by the short period (2±7 days) the flour is stored in the household (Table 16).

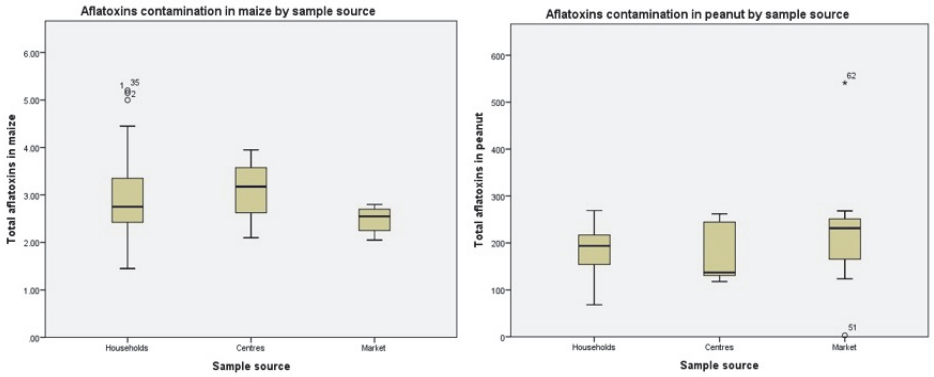


Figure 10. Aflatoxins contamination levels (µg/kg) in maize and peanut flour per households, centres and market in Musanze District in 2015

Aflatoxins exposure in children

From the 24-hour recall results, the median dietary intake of maize flour and peanut flour was 77.8 (10.1-142.2) mg/kg bw and 0.0 (0.0-0.0) mg/kg bw respectively (Table 19). The initial peanut flour intake (g) of children was low, with a minimum and maximum value of 0 and 24 g, before the weight of each child was considered. Thus, the zero values presented in table 19 are not entirely true zero values. On the other hand, some children did not consume any maize or peanut flour in the 24 hours that preceded the interview. Thus, the minimum and maximum intake (mg/kg bw) for maize and peanut flour were 0-4005 and 0-2993 respectively. Maize was consumed in higher quantities because it is a staple crop in the region while on the contrary, peanut flour was consumed in smaller amount by adding it to main dishes or cooked as soup. In our study population, we observed that mothers tend to give more maize flour to older children while younger children are fed peanut flour more. Mothers adopt this feeding pattern probably because maize is a source of energy and offers satiety to older children, while peanut flour is highly nutritious to the young and growing child (Mupunga et al., 2017). There was a significant difference in maize consumption between age groups and stunting status: older children had a significantly higher maize intake than younger children (p <

0.002), and stunted children consumed significantly higher amount of maize flour than their non-stunted counterparts ($p < 0.05$). Although for peanut flour intake there was no significant difference in children age groups and stunting status, younger children aged 5-11 months and 12-17 months, and stunted children, appeared to consume higher amounts of peanut flour. The non-significant result might be due to the overall low intake of peanut flour in the day that preceded the 24-hour recall interview. Children were exposed to aflatoxins from both maize and peanuts. Stunted children had a higher range of exposure (0.0-580.1 μg/kg bw/day) compared to non-stunted children (0.0-205.7 μg/kg bw/day). Our results show that children in the Northern region of Rwanda are constantly exposed to aflatoxins confirming the high and chronic exposure reported in Sub-Saharan Africa (Wagacha & Muthomi, 2008) and the East-African region (Kimanya, 2015). Despite the overall low levels of aflatoxins found in maize flour, the regular and high intake of maize flour by children in the Northern region of Rwanda as a complementary food constantly exposes them to aflatoxins.

Table 19. Maize and peanut flour intake and exposure to aflatoxins in children[†]

	N	Daily maize flour intake (mg/kg bw)	Daily peanut flour intake (mg/kg bw)	AF exposure (µg/kg bw/day)		Total AF exposure (µg/kg bw/day)	Range of total AF exposure (µg/kg bw/day)
				From maize	From peanut		
All children	145	77.8 (10.1-142.2)	0.0 (0.0-0.0)	0.2 (0.0-0.4)	0.0 (0.0-0.0)	0.3 (0.1-1.6)	0.0- 580.1
Age groups (months)							
5-11mo	49	36.8 (0.0-89.7)	0.0 (0.0-0.0)	0.1 (0.0-0.3)	0.0 (0.0-0.0)	0.1 (0.0-0.4)	0.0-580.1
12-17mo	46	64.1 (0.0-113.2)	0.0 (0.0-45.2)	0.2 (0.0-0.3)	0.0 (0.0-9.7)	0.3 (0.0-9.9)	0.0-215.4
18-23mo	35	108.4 (25.4-261.7)	0.0 (0.0-0.0)	0.3 (0.1-0.7)	0.0 (0.0-0.0)	0.3 (0.2-5.5)	0.0-61.4
24-30mo	14	211.5 (42.2-250.5)	0.0 (0.0-0.0)	0.6 (0.1-0.7)	0.0 (0.0-0.0)	0.6 (0.1-0.7)	0.0-5.3
p-value		*0.002**	*0.099*	*0.002**	*0.187*	*0.173*	
Stunting status[‡]							
Stunted	61	89.3 (21.7-208.7)	0.0 (0.0-8.2)	0.1 (0.0-0.3)	0.0 (0.0-0.0)	0.2 (0.0-0.6)	0.0-580.1
Non-stunted	77	44.9 (0.0-119.2)	0.0 (0.0-0.0)	0.3 (0.1-0.6)	0.0 (0.0-1.9)	0.5 (0.1-7.7)	0.0-205.7
p-value		*0.046**	*0.474*	*0.048**	*0.309*	*0.06*	

[†]Values are median and 25th-75th percentiles. AF: aflatoxins. BW: body weight. Mann-Whitney U test was used to compare stunting status and Kruskal-Wallis test was used for age groups. *significant association at *p*-value < 0.05. [‡]Seven children had missing values on their stunting status

The association between aflatoxin exposure and growth status

Table 20 illustrates the outcome of the linear regression modelling of height-for-age with aflatoxins exposure. The crude association between height-for-age and total aflatoxins exposure was not significant (Table 19). However, as shown in table 20, after adjusting for the factors previously found to be associated with stunting in the Northern region of Rwanda (Uwiringiyimana et al., 2018b), aflatoxins exposure in children was negatively associated with height-for-age (p = 0.013). This association is a major finding of this research and shows that not only socio-demographic and nutrition factors are influencing the height-for-age of children but also the quality and safety of complementary flours might be playing a role. The age of children was significantly and negatively associated with height-for-age, especially for age group 18-23 months (p = 0.000) and 24-30 months (p = 0.000). On the other hand, exclusive breastfeeding (p = 0.012), use of deworming tablets (p = 0.000), the BMI of caregivers (p = 0.022) and the dietary zinc intake (p = 0.003) were positively associated with height-for-age. The model adjusted r^2 was higher (r^2= 0.38) compared to the r^2 of the model without aflatoxin exposure (r^2= 0.29) indicating the significant contribution of the aflatoxin exposure in explaining the low height-for-age of children in Musanze District.

Table 20. Regression coefficients from multivariate modelling of aflatoxins exposure in children aged 5-30 months with height-for-age[†]

			95% CI for β	
Variables	β	p-value	Lower bound	Upper bound
Age (months)				
Age group 12-17mo vs 5-11mo	-0.91	0.065	-1.886	0.059
Age group 18-23mo vs 5-11mo	-2.35	0.000	-3.239	-1.458
Age group 24-30mo vs 5-11mo	-2.61	0.000	-3.988	-1.232
Exclusive breastfeeding (yes)	0.68	0.012	0.153	1.202
Use of deworming tablets (yes)	1.87	0.000	1.061	2.677
BMI of caregiver (kg/m2)	0.09	0.022	0.014	0.176
Dietary zinc intake (mg)	2.424	0.003	0.830	4.017
Interaction terms between age groups and energy intake				
Age group 12-17mo*energy intake	-0.002	0.012	-0.005	-0.001
Age group 24-30mo*energy intake	-0.003	0.078	-0.005	0.000
Exposure to aflatoxins (μg/kg bw/day)	-0.005	0.013	-0.010	-0.001

[†]Model adjusted r^2: 0.38. β: Regression coefficient. CI: confidence interval

In a longitudinal study, Gong et al. (2004) found similar results in post-weaning children from Benin. The exposure to aflatoxins was found to negatively affect children's length-for-age over a period of eight months (Gong et al., 2004). It is known that as a child grows up and is introduced to complementary foods, the risk of stunting equally increase due to the poor quality of the complementary foods, inadequate breastfeeding practices and, poor food and water quality (Stewart et al., 2013). Our study indicates that aflatoxins exposure plays a role in the chronic levels of stunting observed in Rwanda, particularly in Musanze. Also, our results indicate that children consuming maize and peanut as part of complementary meals are exposed to high levels of aflatoxins. Although the government of Rwanda has implemented different policies to tackle chronic malnutrition and has seen a reduction in the levels of stunting from 44% in 2010 to 38% in 2015 at the national level (NISR et al., 2015), the levels of stunting are still very high in some

regions compared to the national prevalence. In our view, the exposure to aflatoxins could be a missing link that could explain why stunting levels are not reducing to the desired rate. The reason is that, as stated in a recent government report by the Ministry of Gender and Family Promotion (MIGEPROF, 2018), currently Rwanda does not have a specific policy on food quality and safety management. Thus, the effects of mycotoxins on stunting levels in Rwandan children is almost unknown. Policies to critically control the levels of aflatoxins in flours used for complementary feeding are urgently needed.

Also, as most of the rural households produce the maize that they consume directly or store for a certain period, increased public awareness of local farmers and consumers about the prevention of mould growth before and during storage is recommended. After the 2005 aflatoxin outbreak in Kenya, it was shown that individuals who knew about aflatoxins through the awareness campaign had lower serum aflatoxins levels than those who did not receive the information (Strosnider et al., 2006). Besides, Jolly et al. (2009) found that among Ghanaian health and agricultural professionals, the awareness about aflatoxins contamination in peanuts was significantly associated with the professionals' decision to increase the knowledge about aflatoxins in the society. In Rwanda, there is still a gap in public awareness about fungal contamination and aflatoxins. For example, interviewing vendors of maize flour in local markets, Nishimwe et al. (2017) found that none of the vendors knew about aflatoxins.

Apart from raising awareness, surveillance and monitoring of aflatoxins levels in maize and peanuts are paramount. This study has shown that the use of rapid test method provides a good indicator of aflatoxins levels in maize and peanuts flours. Rapid test methods can be used in Rwanda for monitoring the contamination of flours on the markets. They are very effective, affordable and do not require much training to

operate for testing aflatoxins levels compared to chromatographic methods (Dzantiev et al., 2014). In addition, they can allow for rapid decision-making and interventions during monitoring (Lattanzio et al., 2018).

Our study was not without limitations. Firstly, our results could not estimate the usual intake of maize and peanut flour in children, and the usual aflatoxin exposure as one day 24-hour recall was used. Thus, our study serves to inform and give a snapshot of the maize and peanut intakes of children and their exposure to aflatoxins through the consumption of the flours. Clinical studies are needed to estimate the level of exposure to aflatoxins by using biomarkers such aflatoxin-albumin adducts in serum to improve the estimate of exposure to aflatoxins (Groopman et al., 2008), and to study also the causal relationship between the exposure and stunting. Secondly, our method for analysing aflatoxins is not a gold standard method for determining the absolute levels of aflatoxins in our samples. Thus, the percentage above cut-offs levels of aflatoxins might have some misclassification, and some random error might have been introduced in our model. Thirdly, because our study covered one district, it could not give more insights into the aflatoxin contamination level along the food chain. Future studies on a national level are necessary to know the extent of aflatoxins contamination over the food chain from the farm or silos to households. The results would be useful in the planning and monitoring of the aflatoxin contamination in maize and peanut flours sold on the Rwandan market, which in turn would help to reduce the exposure in children and the general population at large.

3.4 Conclusion

Maize and peanut flours fed to children as part of the weaning diet are a source of high exposure to aflatoxins in Musanze District. Peanut flour particularly contains extremely high levels of aflatoxins, which exceeded

all the maximum tolerable limits in the region and internationally. The present findings show that aflatoxins exposure from maize and peanuts is indeed associated with stunting in children in the Northern Province of Rwanda. The aflatoxins contamination of the complementary flours and the exposure that ensues from their consumption could be the missing link in the reduction of stunting levels in Rwanda. More evidence is needed on the extent of contamination and exposure to aflatoxins in Rwandan children. Policies and programs to reduce and eventually minimize the daily exposure to aflatoxins in children are urgently needed.

Chapter 4 Stunting spatial pattern in Rwanda: an examination of the demographic, socio-economic and environmental determinants[7]

[7] This chapter is based on Uwiringiyimana, V., Amer, S., & Veldkamp, A. Stunting spatial pattern in Rwanda: an examination of the demographic, socio-economic and environmental determinants (Accepted for publication in Geo-Spatial Health Journal).

Abstract

Stunting is recognized as a major public health problem in Rwanda. We aimed to study the demographic, socio-economic and environmental factors determining the spatial pattern of stunting in Rwanda. A cross-sectional study using the data from the 2014-15 Rwanda Demographic and Health Survey and environmental data from external geospatial datasets was conducted. The study population was children less than two years matched with their mothers. A multivariate linear regression model was used to estimate the effects of demographic, socio-economic and biophysical factors, and a proxy measure of aflatoxins exposure on height-for-age. Age of child, height of mothers, secondary education and higher, a child being male and birthweight were associated with height-for-age. After adjusting for demographic and socioeconomic factors, elevation and being served by a market a rural market were also significantly associated with low height-for-age in children. A spatial prediction map examining the stunting pattern revealed how the variability of height-for-age on cluster-level is lost when the stunting prevalence levels are aggregated on a district level. No associations with height-for-age were found with exclusive breastfeeding, use of deworming tablets, improved water source and improved sanitation in the study population. Our study confirms also the influence of environmental factors in determining the height-of-age of children in Rwanda. A consideration of the environmental drivers of anthropometric status is crucial to have a holistic approach to reduce stunting in children.

Keywords: *stunting, environment, height-for-age, spatial pattern, Rwanda*

4.1 Introduction

Stunting among children less than five years remains a public health concern in Sub-Saharan Africa. The East-Africa and the Middle Africa regions have the highest stunting levels of 36.7% and 32.5% respectively (FAO et al., 2017). Stunting or low height-for-age is a complex health problem (Huey & Mehta, 2016) with negative short and long-term effects on a child's health and development. Stunting results in decreased motor and cognitive development, impaired immunity and low education attainment (Dewey & Begum, 2011). In adulthood, stunting leads to lower economic productivity, increases the risk of chronic diseases and lower offspring birthweight (Victora et al., 2008). Evidence has shown that stunting increases tremendously in the first two years of life especially during the complementary feeding period (Black et al., 2008; Victora et al., 2010).

A prolonged lack of adequate and nutritious diet, coupled with recurring infections are the direct causes of stunting. However, a combination of factors underlies the development of stunting in a child. Child-related factors such as child age, lower birth weight, lack of exclusive breastfeeding and inadequate complementary feeding practices are known increase the risk of stunting. Also, stunting is influenced by maternal factors such as short maternal stature, lower education, short birth spacing and poor health before conception; coupled with poor household and family factors such as household income, inadequate water and sanitation, and lower access to health services (Stewart et al., 2013).

Moreover, the biophysical environment which influences household food security and dietary diversity has implications on a child nutritional outcome. The biophysical and geographical factors often studied in the literature include elevation, rainfall, temperature, relative humidity, topography, soil type, aridity, distance to urban areas and

market access through road networks (de Sherbinin, 2011; Nikoi & Anthamatten, 2013). Rainfall, elevation and temperature influence crop production, household food security and dietary diversity, and have been linked to high levels of malnutrition in some regions (Balk et al., 2005). For example, significant differences in stunting levels were found in arid regions of West Africa where drought-resistant crops are grown, compared to humid regions where root crops were dominant (Legg, 2008). Although some research found no consistent relationship between environmental factors and malnutrition (Nikoi & Anthamatten, 2013), biophysical factors directly or indirectly affect the children nutritional outcomes through the impact they exert on agricultural production, household incomes and food availability especially in rural areas among small-holder farms (de Sherbinin, 2011).

Apart from the demographic, socio-economic and biophysical factors, the safety of the food consumed by children has been examined in light of its contribution to stunting. The major challenge many developing countries still face is the dietary exposure to mycotoxins through the food supply chain (Udomkun et al., 2017). Worldwide and in developing countries particularly, mycotoxins constitute a significant threat to achieving the United Nations' sustainable development goal on food security and food safety (Gbashi et al., 2018; Logrieco et al., 2018). Mycotoxins are toxic secondary metabolites produced by a group of fungal species that grow on cereals, nuts and legumes before or after harvest, in the right environmental conditions of humidity, moisture and temperature (Pitt, 2000; Cotty & Jaime-Garcia, 2007). They enter the food chain through contaminated cereals, nuts and their derived products. Because most developing countries rely on subsistence farming and informal local markets, the monitoring and application of regulations for mycotoxins control are often challenging and sometimes non-existent (Gbashi et al., 2018). Evidence has shown that the exposure to mycotoxins for children especially during the weaning period is

deleterious to their growth as it impairs child's immunity and linear growth (Shephard, 2008b; Khlangwiset et al., 2011; Vilcins et al., 2018). Among mycotoxins, the exposure to aflatoxins and fumonisins has been consistently linked to stunting in children (Wild & Gong, 2010; Amuzie et al., 2016). Maize and peanut flours, which are staple foods and thus are fed to children as part of complementary flours in many countries, are among the primary vehicles of aflatoxins and fumonisins exposure (Udomkun et al., 2017).

In the past decade, Rwanda has made remarkable progress in increasing food security (MINAGRI et al., 2016), access to health care (Lu et al., 2016), water and sanitation, and in reducing childhood undernutrition especially wasting (NISR et al., 2015). However, stunting remains high with 38% of children under five being classified as stunted (NISR et al., 2015). It is estimated that Rwanda loses 11.5% of its gross domestic product due to child undernutrition (AUC & NEPAD, 2013). In 2013, the country had set a target of reducing child stunting to 24.5% by 2018 (MINALOC et al., 2014b). However, this target is still hard to attain. Recognising the complexity of stunting, Rwanda adopted a multisectoral approach focusing on early childhood development, improving water and sanitation, and increasing social protection (MIGEPROF, 2018). On the other hand, as mentioned in the recent Rwanda Strategic Review of Food and Nutrition Security report, the factors leading to the high levels of stunting in some regions in Rwanda remain unclear (MIGEPROF, 2018). Also, the contribution of mycotoxins exposure to the high levels of stunting observed in Rwanda remains a domain that is not well investigated. Lack of national level data on mycotoxins occurrence in the food chain, as a result of lack of funds or research facilities, is a serious challenge to mycotoxin research in most developing countries (Gbashi et al., 2018), and Rwanda is not spared. Consequently, the impact of the exposure to mycotoxins on stunting levels remains unknown. To understand the local determinants of

stunting, we conducted research in the northern region of Rwanda, which in 2010 was among the districts with high levels of stunting (Uwiringiyimana et al., 2018b). In addition, by examining the impact of aflatoxins exposure on children's height-for-age, we found that the exposure to aflatoxins from complementary flours was associated with low child height-for-age (Uwiringiyimana, Ocke, et al.). It is in this regard, that the present study takes a holistic approach and examines the determinants of stunting in Rwanda by taking considering not only the usual demographic and socio-economic factors known to influence stunting but also biophysical factors and mycotoxins exposure.

However, due the lack of spatially explicit, nationally representative data on mycotoxins in maize and peanut flours in Rwanda, examining their impact on child's nutritional status on a spatial scale is a challenge. To overcome this lack of data, we considered the food supply chain of maize and peanut flours in Rwanda. Most households (70%) in Rwanda get their complementary flours from markets (MINAGRI et al., 2016). In the local study conducted in the Northern region of Rwanda (Uwiringiyimana, Ocke, et al.), nearly all households surveyed (99%) obtained their peanut flour from the markets, and there was an observed difference in the aflatoxins levels of flours from the main market and local centres. As it is known that aflatoxins production increases along the food supply chain and during storage (Hell & Mutegi, 2011), the population that purchase the flours, from markets at the end point of the food supply chain, have a higher risk of exposure to aflatoxins (Kamika et al., 2016). This is because the environmental conditions that exist in Sub-Saharan Africa such as the climate, temperature and humidity are very conducive for the growth of fungi and aflatoxins production, particularly during post-harvest handling and storage (Gnonlonfin et al., 2013). Thus, in this study, we hypothesised that household clusters that are supplied by markets located at the lower end of the food supply chain would have an increased risk of being exposed to highly

contaminated flours, and thus will be more prone to low height-for-age. Thus, the objectives of this study were first, to examine the demographic and socio-economic factors associated with the observed height-for-age variability in Rwanda. Second, controlling for the demographic and socio-economic factors, we studied the biophysical factors affecting height-for-age variability and lastly, adjusting for the previous factors, we investigated whether obtaining flours from the lower end of the food supply chain was associated with childhood height-for-age? In this present study, in addition to socio-economic factors, the consideration of biophysical factors and a proxy measure of the exposure to aflatoxins renders our analysis more robust, and enables us to make a spatial explicit prediction of stunting occurrence.

4.2 Materials and methods

Study area

Rwanda is a country located in East-Africa with Tanzania to the East, Uganda to the North, Congo to the West and Burundi to the South. About 61.5% of Rwanda lies above 1.500 meters above sea level, giving the country a temperate climate (Mendelsohn et al., 2016). Rwanda has four provinces, 30 districts, 416 sectors, 2,148 cells and 14,837 villages. The population of Rwanda is about 11 million, among which 1,5 million are under five years (NISR & MINECOFIN, 2012). Figure 11 shows the spatial variability of stunting among the 30 districts of Rwanda.

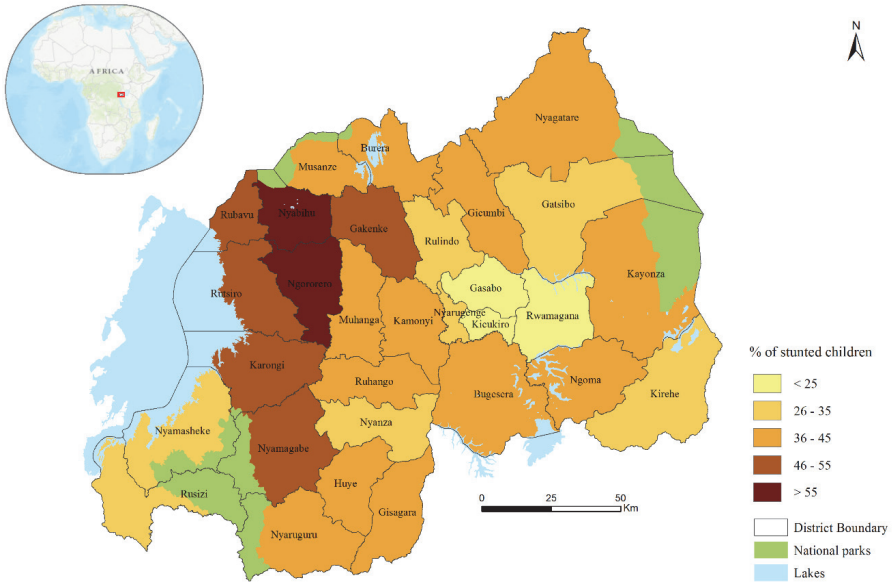


Figure 11. Stunting prevalence (%) per district in Rwanda in 2015 (based on DHS, 2015), with the Africa map showing Rwanda location.

Data

This study is based on the household cluster level demographic and socio-economic data from the 2014-15 Rwanda Demographic and Health Survey (DHS) (NISR et al., 2015). The survey was conducted in a two-stage sample design. In the first stage, clusters within enumeration areas that represent the 30 districts of Rwanda were selected. Clusters are the primary sampling units and they contain on average 100 and 300 households from which 20 to 30 households are randomly selected for survey participation. In Rwanda, a total of 492 clusters were selected, 113 were located in urban areas, and 379 were located in rural areas. The second stage involved the systematic sampling of 26 households within each cluster, making up a sample of 12,792 households on a national level. From a sub-sample consisting of 50% of the households, indices of anthropometric status (child height and weight), individual mother and

household characteristics were recorded for 3,813 children less than five years. At the end of the survey, 1467 children aged less than two years had valid height-for-age measurements, matched with their mothers' characteristics. This study was based on this sample. All the demographic and socio-economic variables used were extracted from the 2014-15 DHS database for Rwanda, taking into account the survey weights, the variables definition and the denominators (Rutstein & Rojas, 2006).

Demographic and socio-economic data

The outcome variable was the height-for-age z-scores (HAZ) of children less than two years. Height-for-age is a measure of stunting or low height-for-age and is an indication of a prolonged nutrient deficit in children resulting in linear growth retardation (de Onis & Branca, 2016). Stunting is indicated by a z-score less than -2 standard deviations (SDs) below the median of the World Health Organization (WHO) reference population (Onis, 2007). The independent demographic and socio-economic variables were:

For every child, age, sex, birthweight, exclusive breastfeeding, the use of deworming tablets in the last six months, and the presence of diarrhoea in the two weeks preceding the survey.

For every mother, height, body mass index (BMI), highest education level and the preceding birth interval were considered. Mother's highest education level categorised mothers as having no education, primary education, secondary education or higher. The preceding birth interval represented the difference in months between the current birth and the previous birth, counting twins as one birth. The socio-economic variables used were wealth index, type of residence, improved source of water, improved sanitation or toilet facility and access to health care facility. The wealth index is a composite measure of a household's living standard or economic status. It is estimated using data on household's

ownership of selected assets such as television, and bicycles; materials used for housing construction; and types of water access and sanitation facilities (NISR et al., 2015).

Environmental data

The biophysical variables considered in the study were elevation, slope, temperature, rainfall and relative humidity. Table 21 displays the description of the environmental data. The elevation was produced from a digital elevation map (DEM) created by the Rwanda National Land Use and Development Master Plan Project through the Rwanda Natural Resources Authority. The slope was calculated from the DEM. The climatic factors namely temperature, rainfall, and relative humidity were measured from a network of 183 meteorological stations throughout Rwanda (Nyandwi et al., 2016). The annual average of these factors was measured for most stations using 60 years records starting from 1950 to 2010 by the Rwanda Meteorological Agency. After, the averages were interpolated for the whole country using thin-plate smoothing spline algorithm and saved as raster datasets with a 10-meter cell size (Nyandwi et al., 2016).

Table 21. Description of environmental variables

Data type	Variables	Description
Topographic	Elevation and slope	The elevation map was created from a DEM and further used to generate the slope using ArcGIS. Both the raster files were created at a high spatial resolution with a cell size of 10m
Climatic	Temperature, rainfall, and relative humidity	Annual means measured at the meteorological weather stations, and interpolated and resampled to a cell size of 10m

Merging environmental data and DHS data

To merge the environmental data with DHS data, each biophysical factor was extracted per household cluster using zonal statistics in ArcGIS Desktop, Release 10.4 (ESRI, Redlands, CA, USA). Consideration was made, however, by taking into account the geographical displacement of the cluster done by DHS for every household survey (Burgert et al., 2013a). For protecting the confidentiality of survey participants, DHS displaces the GPS coordinates of urban clusters up to two kilometres (0-2 Km) and of rural clusters up to five kilometres (0-5 Km). Thus, before extracting the biophysical factors per cluster, we created a buffer of five kilometres around all rural clusters and a buffer of two kilometres around all urban clusters. After, using the zonal statistics tool, the mean value of each environmental variable was extracted.

Classification of household clusters

As the environmental conditions in the tropics are known to be very conducive to the production of mycotoxins (Gbashi et al., 2018), and given that aflatoxins contamination increases along the food supply chain due to poor post-harvest management (Kamika et al., 2016), we considered household clusters that would obtain their flour from markets at the lower end of the food supply chain as being at risk of exposure to higher aflatoxins levels from complementary flours compared to household clusters that obtain their flour at the start of the food supply chain. Because the food supply chain in Rwanda is organized in a hierarchy of markets, from main markets to regional and local markets, we used different data layers to classify clusters according to whether they are served by an urban market, a rural market only, or are not served by neither an urban nor a rural market. Household clusters served by an urban market were considered as clusters that obtain their flours from markets at the top of the food supply chain, and thus have the least risk to aflatoxins exposure; clusters served by only a

rural market were considered as clusters that obtain their flours from markets at the intermediate level of the food supply chain and thus have an increased risk of exposure compared to the first category; and finally clusters neither served by an urban or rural market were considered as clusters that obtain flours from local shops at the end of the food supply chain. These household clusters would have the highest risk of being exposed to aflatoxins.

The following data layers were used to classify clusters: the road network of Rwanda, the locations of markets and the existing boundary of urban areas. The national road network was produced by the Rwanda Land Management and Use Authority (RLMUA) within the land cadastral project in 2009-2010 and categorises roads into national, district and other roads. Also, the existing boundary of urban areas in Rwanda was delineated by RLMUA during its land demarcation project for the production of urban master plans of the districts in Rwanda. The location of markets was produced by the National Institute of Statistics of Rwanda (NISR) by collecting the location of each market on the ground. A summary of the process followed to compute the distance variables is shown in figure 12. We computed the distance from each household cluster to the closest urban or rural market in ArcGIS Desktop, Release 10.4 (ESRI, Redlands, CA, USA).

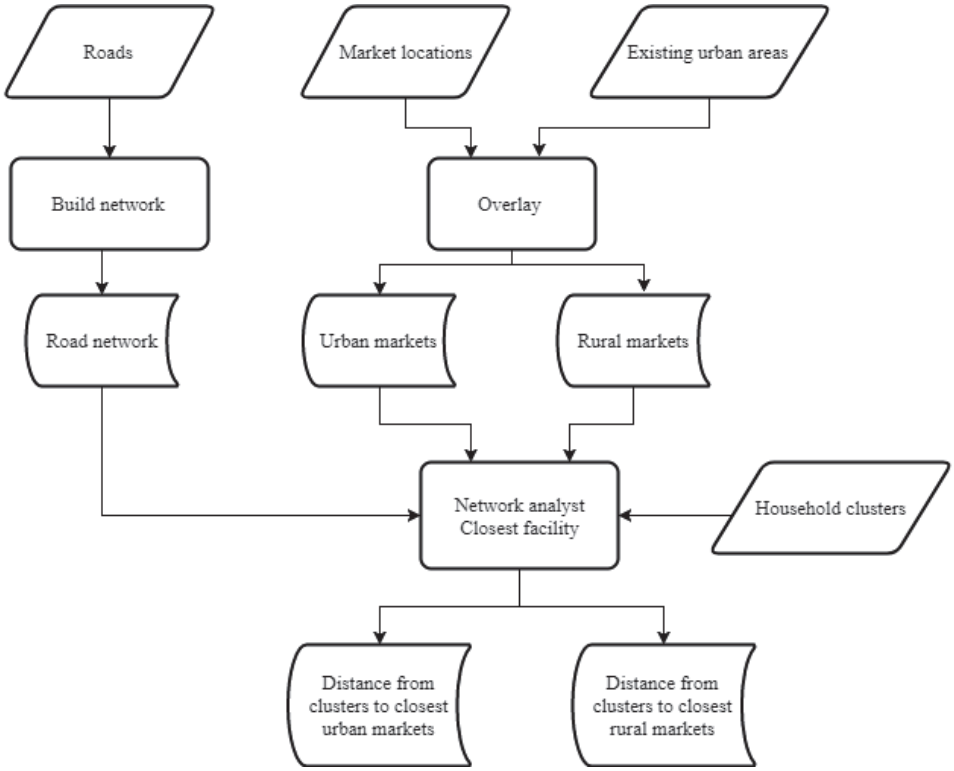


Figure 12. Flowchart for the calculation of the distance to markets

First, to prepare the data for network analysis, a road network was built using the road layer. Second, the markets layer was overlaid with the existing boundary of urban areas, and markets were extracted and categorised as urban or rural. This categorization was done to take into account the fact that main markets in urban areas supply flours to rural markets. Third, using network analyst, two distances variables were calculated from each household cluster to the closest urban and rural markets.

Finally the resulting distance variables were imported in IBM SPSS Statistics for Windows, version 24.0 (IBM Corp., Armonk, NY, USA) for further processing. Because of the geographic displacement of the household clusters done by DHS to protect the confidentiality of survey respondents, using a continuous distance variable from a household

cluster to any closest facility as we computed could introduce a bias in the measurement (Burgert et al., 2013b). To reduce the bias, categorizing the distance is recommended (Burgert et al., 2013b). Thus, we used two categories of 5 Km and 10 Km distances to the markets. These two cut-offs were chosen to consider a walkable distance from a household cluster to a closest market and also because they take into account the geographical displacement of both the urban and the rural household clusters. The distance variables were used to classify the household clusters into the three categories depending on where they would obtain their flours, all within a 5 Km or 10 Km radius: (1) household clusters served by an urban market; (2) household clusters served only by a rural market; and (3) household clusters neither served by an urban market nor a rural market.

Statistical analysis

All variables were imported to IBM SPSS Statistics for Windows, version 24.0 (IBM Corp., Armonk, NY, USA) for statistical analysis. Descriptive statistics was run on all variables. Mean, median and standard deviation were reported for continuous variables. Percentages were displayed for discrete variables. Maps of the independent variables aggregated per household cluster were produced to visualise their spatial pattern. Correlation between the anthropometric status and the independent variables were ran on all variables to study the linear relationship with height-for-age. A hierarchical stepwise linear regression model was applied to determine the factors that were significant determinants of HAZ. Three hierarchical stepwise models were run, with demographic and socio-economic factors that affect height-for-age entered first. The second block consisted of environmental variables, and in the third block, the household clusters classification variables, based on the type of market that serve each cluster were included. The independent variables retained in the final model were all significant. A p-value of < 0.05 indicated statistical significance. Given that most regression analyses have been criticised of overlooking or not correcting for the

spatial dependence which biases the results of the regression models (Voss et al., 2006; de Sherbinin, 2011), we checked for possible spatial autocorrelation in our analyses. Thus, the results of the final model were validated by visualising the pattern of the residuals in ArcGIS Desktop, Release 10.4 (ESRI, Redlands, CA, USA), and running a spatial autocorrelation analysis with Global Moran's I statistic to assess the non-spatial clustering of the model residuals. Spatial autocorrelation indicates that the errors in the model are not independent (Voss et al., 2006). When spatial autocorrelation is present, it reduces the standard errors of the estimates, increases the t-values and reduces the *p*-value thereby leading to a bias in the model (Voss et al., 2006). Moran's I is similar to Pearson's correlation coefficient, and has values that range from -1 to 1, with positive values indicating high values surrounded by high values and, negative results showing spatial randomness. A prediction map based on the model predicted height-for-age z-scores per household cluster and per district was produced using natural breaks.

4.3 Results

Sociodemographic characteristics of study population

The descriptive characteristics of the study population is presented in Table 22. The mean (SD) height-for-age was -1.2 (1.5) and stunting prevalence was 30.2 %. The mean (SD) child age and mean (SD) birthweight were 11(7) and 3.3 (0.6) respectively. The population of male and female children was 49.9% and 50.1% respectively. A small percentage of children (25.3%) had been exclusively breastfed. About half of them had received deworming tablets in the past six months and 17% had had diarrhoea in the last two weeks. Mother's body mass index ranged from underweight (12.4) to obese (45.5). The average difference between the current birth and the previous birth was 11 months. The majority of mothers had primary education (71.8%), while only 2.4% had

higher education than secondary school. About 70% of all households had access to an improved water source and improved sanitation.

Table 22. Descriptive statistics of dependent and independent variables used in the study

Variable	Mean	SD	N	%
Continuous				
Height-for-age (HAZ)	-1.2	1.5	1467	
Child age (months)	11	7.0	1514	
Child birthweight (kg)	3.3	0.6	1509	
Mother's BMI (kg/m^2)	23.1	3.5	1509	
Mother's height (cm)	156.9	6.1	1509	
Birth index (months)	11.4	6.6	3122	
Wealth index	-0.1	0.8	3122	
Elevation (meters)	1702	348.0		
Slope (in degrees)	11.7	10.9		
Rainfall (mm)	1208	259.0		
Relative humidity	73.9	3.6		
Categorical				
Stunting			1467	30.2
Sex			3122	
Female				50.1
Male				49.9
Exclusive breastfeeding			2972	
Yes				25.3
Mother's education			3122	
No education				13.3
Primary education				71.8
Secondary education				12.5
Higher				2.4
Improved water source*			3074	
Yes				72.5
Improved sanitation°			3074	
Yes				70.2
Deworming tablets use in the last six months			3116	
Yes				48.6
Diarrhea in the last two weeks			3122	
Yes				17.0

*Improved water source includes water piped into a dwelling, public tap/standpipe, tube well or borehole, protected well, protected spring and rainwater.

°Improved sanitation includes flush/pour flush to a piped sewer system, flush/pour flush to a septic tank, flush/pour flush to a pit latrine, ventilated improved pit latrine, pit latrine with slab, composting toilet. Shared improved facilities are also included.

Source: DHS Rwanda 2014-2015 and authors' own calculations.

Figure 13 displays the spatial variability existing within the demographic and socio-economic variables per cluster. The prevalence per each variable is shown. The high percentage of stunting is found more in the North, West and South-Eastern part of Rwanda and varies from 0% to 100% in some clusters (panel a). Exclusive breastfeeding of children less than six months was generally below 75% (panel b). As expected, mothers with secondary education and higher mostly lived in Kigali (panel c). On the other hand, the BMI of mothers were uniformly spread across the country (panel d).

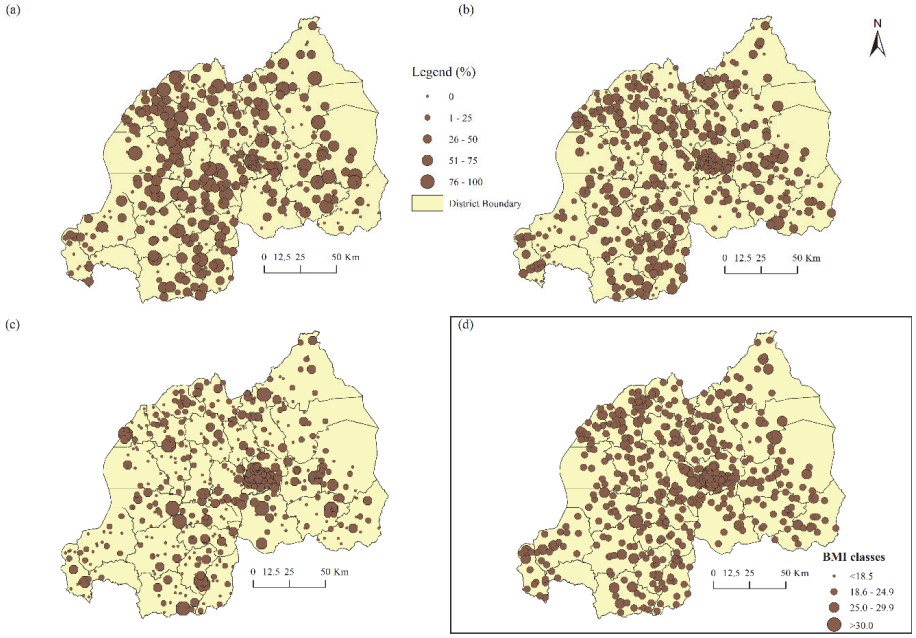


Figure 13. Prevalence rate (%) of DHS variables per cluster. Prevalence rate of stunting (a), Percentage of exclusive breastfeeding (b), and secondary and other higher education per mother (c). Map (d) shows the mothers' body mass index (BMI) classes per cluster.

As shown in figure 14, the percentage of children that received deworming tablets in the last six months (panel a) was higher across the country as opposed to the prevalence of diarrhoea (panel b). The use of non-improved water source (panel c) in the households was more

pronounced in the East, North and some regions in the South. On the other hand, the use of non-improved sanitation (panel d) was evenly spread across the four provinces of Rwanda.

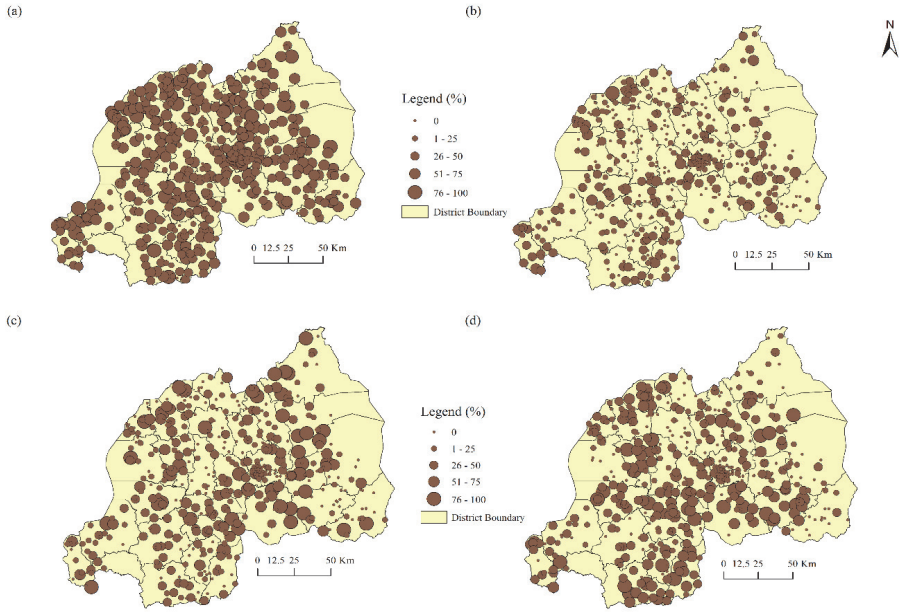


Figure 14. Prevalence rate (%) of DHS variables per cluster. Percentage of deworming tablets use in the last six months (a), presence of diarrhea in the last two weeks (b), non-improved water source in the households (c) and non-improved sanitation in the households (d).

Classification of households clusters

The household clusters were classified according to the likeliness of being served by markets located at the top, intermediate and lower end of the food supply chain. This was done first estimating the distance from each cluster to the closest urban or rural market. Table 23 shows the results from the household cluster classification. Household clusters that were served by an urban market within a 5 Km and a 10 Km radius were 21% and 32 % respectively. Household clusters served only by a rural market were 39% and 61% within the 5 Km and 10 Km respectively. Household clusters served by neither an urban nor a rural market within 5 Km were 40%, and 7% within 10 Km.

Table 23. Household clusters classification

Variable	N (%)	
	5 Km	10 Km
Distance categories		
Household clusters served by an urban market	106 (21)	158 (32)
Household clusters served only by a rural market	190 (39)	300 (61)
Household clusters served by neither an urban or rural market	196 (40)	34 (7)

N: sample size; %: percentage; Km: kilometer

Figure 15 shows the spatial distribution of the household clusters as served by an urban market or a rural market within a 5 Km (panel a) and 10 Km radius (panel b). The majority of household clusters that were served by a market at the top of the food supply chain were located in urban areas. According to our assumption of increased aflatoxins contamination along the food supply chain, these household clusters were considered as clusters with the least risk of exposure to higher aflatoxins level. Household clusters served by markets at the intermediate level in the food supply chain were mostly located in rural areas; and thus they will be more exposed to aflatoxins compared to clusters located in the urban areas, assuming the rural markets get their supply from the urban markets. Lastly, the household clusters neither served by an urban market nor a rural market within a 5 Km and 10 Km radius, which is the third category, will be potentially the most exposed to higher levels of aflatoxins.

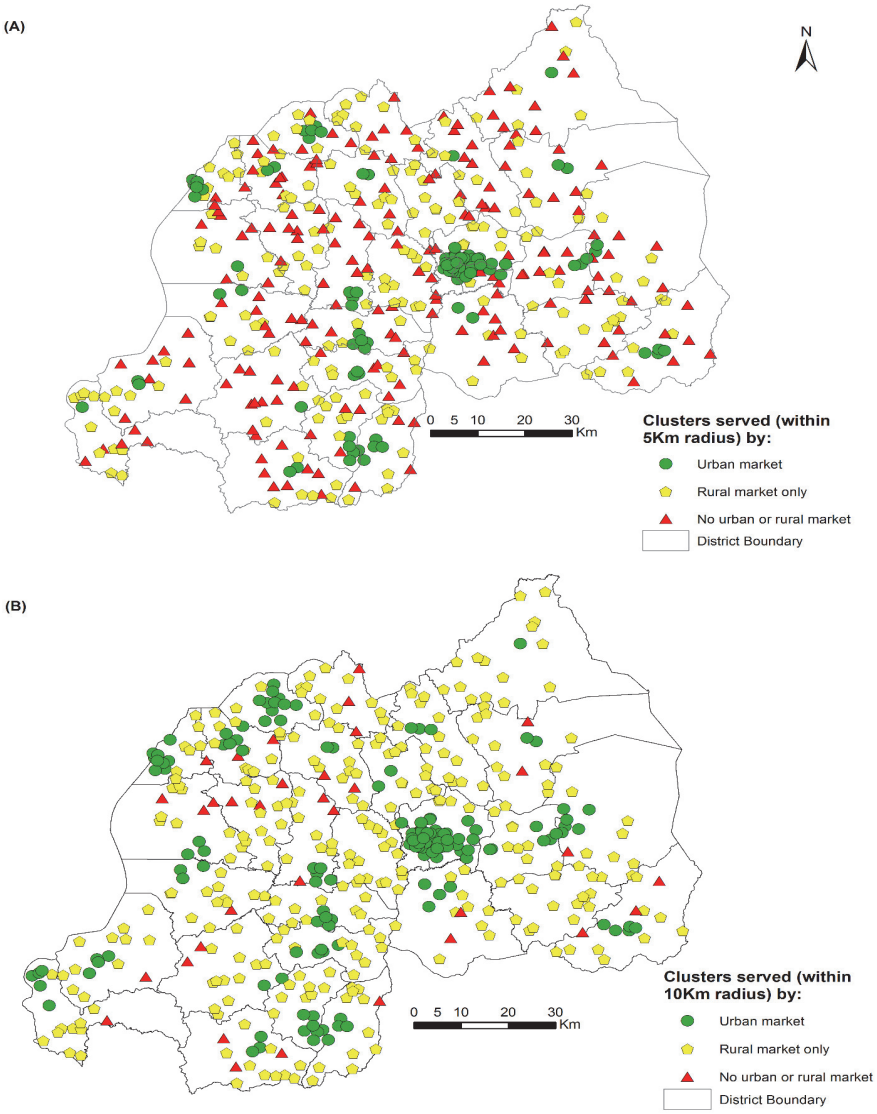


Figure 15. Distribution of household clusters as served by the three categories of markets in Rwanda, within a 5 Km distance (a) and within a 10 Km distance (b).

Determinants of height-for-age

Table 24 shows the results of the hierarchical linear regression model. The child age (p=0.000), mother's height (p=0.000), secondary education (p=0.003), being male (p=0.001), children's birthweight (p=0.014) were

significantly associated with height-for-age. From the biophysical factors, elevation (p=0.000) was a significant predictor of height-for-age in children. Being served by a rural market within 10 Km radius (p=0.041) was negatively associated with the height-for-age in children. The HAZ significantly decreased as a cluster changed from being served by an urban market to being served by a rural market within a 10 Km radius. The adjusted R^2 of the final model was 0.27, implying that 27% of the total variability in stunting can be explained by this significant empirical model.

Table 24. Regression coefficients of the socio-economic, environmental and accessibility factors on height-for-age (HAZ)

Variables	Unstandardized coefficients		Standardized coefficients	95% CI
	B	Standard error	Beta	Lower, Upper
Child's age (months)	-0.08**	0.01	-0.328	-0.102, -0.062
Mother's height (cm)	0.06**	0.01	0.223	0.036, 0.078
Secondary education and higher	0.01*	0.00	0.128	0.002, 0.010
Sex of child-male	-0.01*	0.00	-0.132	-0.010, -0.002
Child's birthweight (kg)	0.32*	0.13	0.102	0.065, 0.583
Elevation (meters)	-0.00**	0.00	-0.207	-0.001, 0.000
Cluster served by an urban vs a rural market	-0.18*	0.09	-0.086	-0.351, -0.007

CI: Confidence interval. *P<0.05, **P<0.001. Adjusted R^2 of the model is 0.27.

Figure 16 shows the results of the spatial autocorrelation analysis with residuals on the x-axis and the lagged residuals on the y-axis. The lagged residuals represent the sum of weighted residuals of neighbouring household clusters. Moran's I was -0.01 with a z-score of -0.73 and a p-value of 0.29. The non-significance of the test indicates that there is no spatial autocorrelation among residuals, thereby confirming their independence.

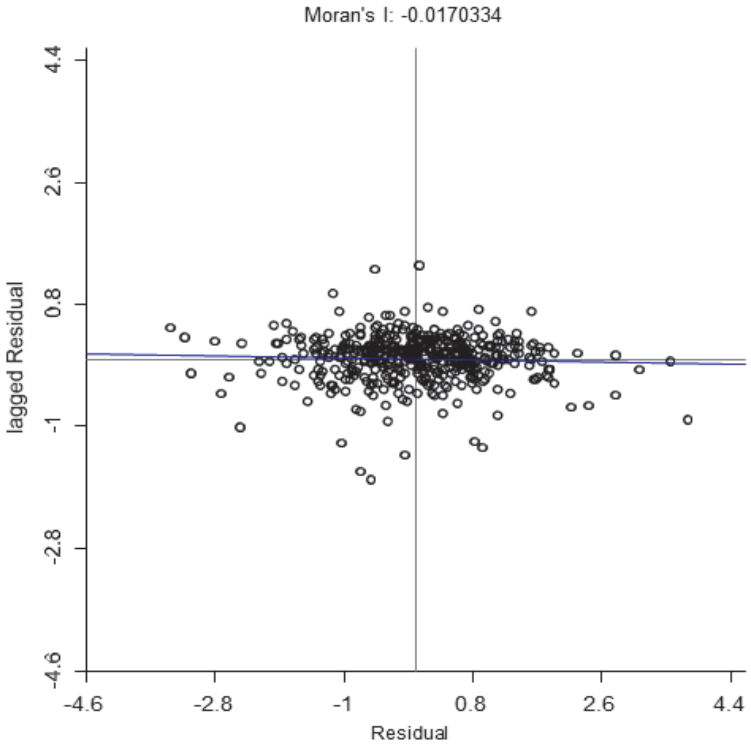


Figure 16. Moran's I for the residuals.

Figure 17 panel b shows the spatial pattern of the predicted values of HAZ in Rwanda, based on the model presented in table 23, with the same mean HAZ aggregated on a district level. Overall, the central region of Rwanda has normal height-for-age values in contrast to the Northern and the Western regions that have mostly low height-for-age values. The aggregation to a district level, however, overshadows the clusters with higher HAZ, which are surrounded by clusters with lower HAZ. The same is also observed from the comparison made with the commonly reported stunting prevalence per district (figure 17 panel a). Compared to figure 11, the spatial variability in HAZ is lost due to data aggregation at the district levels.

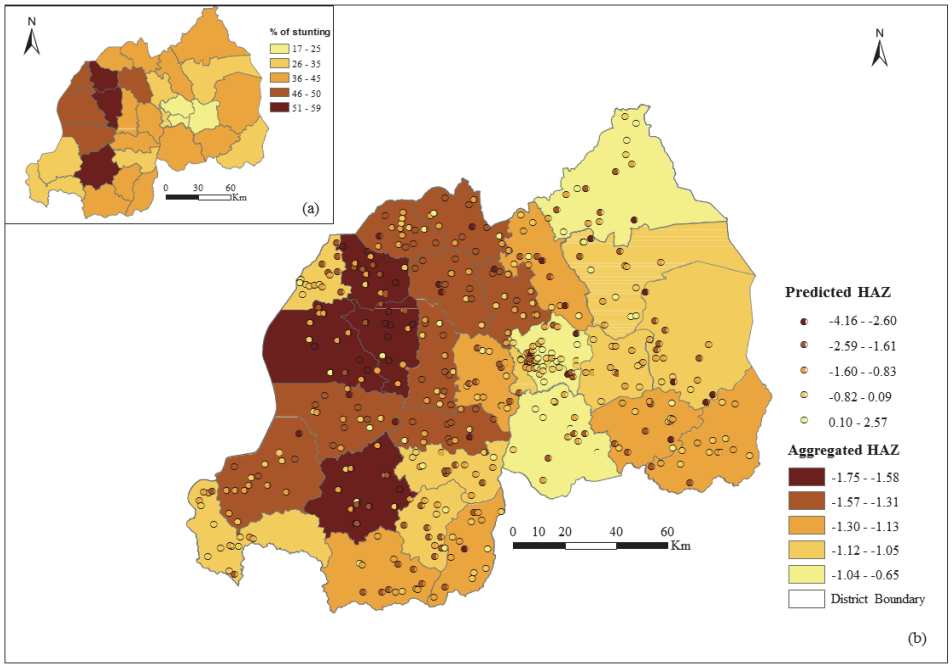


Figure 17. Model-predicted height-for-age z-scores per cluster (b) with the same values shown in the background aggregated on a district level, compared to the prevalence of stunting (a) per district

4.4 Discussion

We studied the determinants of the spatial pattern of height-for-age on household cluster level in Rwanda. We considered demographic and socio-economic variables, environmental variables with a proxy variable for aflatoxins exposure in children through the supply of complementary flours to households. Stunting prevalence in children was 30.2%, which according to the new stunting prevalence thresholds is considered 'very high' (de Onis et al., 2019). The child and mother factors associated with height-for-age were child age, mother's height, secondary education and higher, a child being male, and child's birthweight. The direction of the relationship for each covariate was as expected. First, the age of children was negatively associated with height-for-age. This result confirms the existing knowledge that as a child increases in age, the risk of stunting (or decreased height-for-age)

increases (Dewey & Huffman, 2009). Secondly, the mother's height was positively associated with the height-for-age of children, highlighting the importance of the impact of maternal nutrition on the anthropometric status of a child. Maternal short stature is known to be a high-risk factor of intra-uterine growth restriction in the developing foetus, which has been linked to childhood stunting (Black et al., 2013).

We found that having a secondary education and higher was positively associated with the height-for-age in children. In Uganda, similar results were found where children from mothers educated above primary school had a lower risk of stunting (Wamani et al., 2007). Maternal education has been often linked to lower height-for-age of children (Chopra, 2006; Sakisaka et al., 2006), also paternal education was shown to have the same influence. In a study conducted in Indonesia and Bangladesh, formal education of both mothers and fathers was found to be a strong determinant of childhood stunting (Semba et al., 2008). In this study, we did not test the influence of parental education due to the lack of available data. On the other hand, as Semba et al. (2008) highlighted, both maternal and paternal formal education can result in better caregiving practices which can reduce the risk of childhood stunting.

Gender and child birthweight are also known to be determinants of childhood stunting. In our study, a child being male was negatively associated with height-for-age. Previous Rwanda DHS surveys (INSR & Macro, 2006; NISR et al., 2010), showed similar patterns, with a notable difference in the prevalence of stunting among boys and girls: 47.4% boys were stunted compared to 41.4% stunted girls in the RDHS 2010, and in RDHS 2005, 46.3% boys were stunted compared to 44.4% stunted girls. In a study on sex differences in the nutritional status of HIV-exposed children in Rwanda, Condo et al. (2015) found significant differences in stunting status, with male children being more stunted

than their female counterparts. However, in multivariate analysis, the authors did not find a significant difference in feeding practices between males and females children (Condo et al., 2015). As for child's birthweight, our findings showed that an increase in a child's birthweight was positively associated with an increase in height-for-age. Research has shown that children who are born with normal weight are more protected against infections and risk of mortality compared to children born with a low birthweight (Black et al., 2013).

Although exclusive breastfeeding, use of deworming tablets, access to improved water source and sanitation of the household are known to determine height-for-age (Stewart et al., 2013), we did not find any significant association of these covariates with height-for-age. The bivariate correlation was significant, however upon considering the variables in multivariate regression, the association was not significant. The same non-significant association was observed for preceding birth interval, mothers' BMI, and type of residence. Studying the risk factors associated with underweight using the 2010 Rwanda DHS data, Mukabutera et al. (2016) found that for children being male, having a fever in two weeks before the survey, being part of multiple births compared to a first single birth increased the risk of underweight. Also, for mothers, having a low BMI, no education or primary education compared to secondary education or higher, and being attended by an unskilled provider during the last delivery were associated with higher odds of underweight in children (Mukabutera et al., 2016).

Among the biophysical factors, elevation of a household cluster was negatively associated with the height-for-age of children. The robustness of the elevation covariate was consistent throughout the analysis. Similarly, Dang et al. (2004) found that children living in mountainous regions were found to have a high risk of becoming stunted than children who lived in lowlands. Elevation could have a direct effect on

height-for-age, but its effect is probably more mediated through the food security and accessibility to health care services. In the comprehensive food security and vulnerability analysis survey conducted in Rwanda in 2015, households living in high altitude areas were found to be more vulnerable to food insecurity, and their children were also found to be more stunted as compared to those living in the rest of the country (MINAGRI et al., 2016). On the other hand, in this study we tested the accessibility to health care services, and did not find a significant effect between the distance to health facilities and height-for-age. This is probably because the health care services delivery in Rwanda is well organized with a universal health care system and thus equally accessible to the majority of the population (Binagwaho, Farmer, et al., 2014). We tested other environmental factors such as rainfall, relative humidity and slope. These covariates were not significant in the final model, because their explanatory power of height-for-age was very low. However, this non-significant result might result from the cross-sectional nature of our study. Because apart from slope which although not significant can be said to be captured in the elevation variable, rainfall and relative humidity are factors that naturally change in space and time, especially according to seasons. Thus, considering seasonality might reveal the impact these factors can have on the anthropometric status of children. Skoufias and Vinha (2012) analysed the impact of weather shocks on child height in rural Mexico from 1951 to 1985; and found that after positive rainfall shocks, defined as one or two standard deviations more than the average 1951-1985 rainfall, children were shorter regardless of their location or altitude. Negative temperature shocks, defined as one or two standard deviations less than the average 1951-1985 temperature, also had a negative impact on child's height but only for high altitude areas and central and southern parts of Mexico (Skoufias & Vinha, 2012).

Household clusters that were served by rural markets within a distance of 10 Km had a significant negative association with height-for-age compared to clusters that were served by urban markets. This association had the second highest effect on the height-for-age of children after the children's birthweight regardless of the direction of the relationship. This result verified our hypothesis that being served by markets located at the lower end of the food supply chain could be a proxy for aflatoxins exposure, given that most households in Rwanda get their complementary flours from markets at different levels in the food supply chain. However, caution should be taken with the interpretation of this finding and its practical meaning. This is because although we used the distance categories, which according to DHS recommendations (Burgert et al., 2013b) can reduce the bias introduced in the model due to the displacement of households' clusters, the distance variables might still not represent the reality on the ground. Also, in reality, the dynamics of aflatoxins production along the food supply are complex and thus, our hypothesis requires further validation by studying the occurrence of aflatoxins along the food supply chain and quantifying the contamination levels in Rwanda. On the other hand, due to the lack of explicitly national spatial data on aflatoxins in foods, which is the case in many developing countries, our approach could be applied to conduct a spatial analysis of the influence of aflatoxins on stunting levels on a country scale.

The use of the Rwanda DHS 2014-15 data had several strengths. First, we analysed matched children and mother characteristics, thus increasing the robustness of the analysis. Second, the survey provided a broad range of explanatory variables of good quality data with a high response rate. Third, the availability of spatial data enabled us to analyse the determinants of stunting on a household cluster-level, which to the best of our knowledge, has not been done before in the Rwandan context. Also, using the household clusters enabled us to take into

account the spatial variability of the covariates, visualise the spatial distribution of height-for-age on a high resolution, control for spatial autocorrelation, and finally make a spatial prediction map for height-for-age. Previous spatial studies in Rwanda concentrated on examining the spatial pattern of other public health issues such as malaria (Bizimana et al., 2016), schistosomiasis (Nyandwi, Veldkamp, Osei, et al., 2017), however studying stunting on a spatial level is still an area that needs more attention. Our study had some limitations. Although for the extraction of environmental factors and the estimation of household exposure to aflatoxins, the displacement of the household clusters was taken into account; there will be a margin of error as the true locations of clusters are unknown (Burgert et al., 2013a). Also, the analysis on household clusters assumes that the covariates are the same across the clustering of households. However, naturally there is some variability that inherently exists within household clusters. Also, our approach to estimating the exposure to aflatoxins using the proxy of for aflatoxins exposure in children through the food supply chain of complementary flours to households would not always mean that buying flours from markets at the intermediate or lower end of the food supply chain necessarily translate into higher exposure to aflatoxins. This is because of the complex intrinsic and extrinsic factors that interplay on the one hand for fungi to be present in a food commodity and on the other hand, for aflatoxins to be produced (Sanchis & Magan, 2004; Sakisaka et al., 2006). Also, our scenario considered only the hierarchy of markets as the source of exposure, which does not take into account the exposure experienced by households that produce and store maize for own consumption.

4.5 Conclusion

Our study confirms not only the usual effect of child and mother factors on height-for-age but also shows the influence of environmental factors in determining the height-of-age of children in Rwanda. Elevation and

being served by markets at the lowered end of the food supply chain were significantly associated with low height-for-age. Thus, an understanding and consideration of the environmental drivers of stunting is crucial in order to have a holistic approach in addressing low height-for-age in children under five years. Our use of household clusters shows the variability of stunting across the study area. In most published studies, the analysis is generally conducted on a household level with no spatial component considered. Although this approach provides valuable insights into the determinants of stunting on individual and household levels, most governmental interventions are targeted on a regional scale. Future research should focus on studying in depth the clustering observed in the height-for-age measure, to better understand the individual determinants of stunting on a finer scale. Conducting the analysis on such a high resolution could shed light on probably some overlooked yet determining correlates of stunting and overall, it would also result in better geographically targeted interventions and the prioritisation of the affected areas. Also, an examination of the temporal change in hotspots of stunting during the past years to understand the areal change in the distribution of stunting by using the publicly available spatial correlates should be considered. For Africa and Rwanda specifically, there is a need of more interdisciplinary research, incorporating geographical information system (GIS) applications in understanding the complexity of stunting, to complement the commonly applied analyses on an individual or household level in the nutrition or social science fields. Lastly, there is a tremendous data gap to be filled on the extent of mycotoxin exposure on a national level and how this affects linear growth, not only on an individual level but also on a regional and national scale.

Chapter 5 Bayesian geostatistical modelling of stunting in Rwanda: risk factors and spatially explicit residual stunting burden[8]

[8] This chapter is based on Uwiringiyimana, V., Osei Frank B., Amer, S., & Veldkamp, A. Bayesian geostatistical modelling of stunting in Rwanda: risk factors and spatially explicit residual stunting burden (Submitted to BMC Public Health).

Abstract

Stunting is still a significant public health issue in Rwanda and its prevalence varies spatially on a local scale. We aimed to apply Bayesian geostatistical modelling to study the spatial pattern of stunting in children less than five years and its anthropometric socioeconomic and demographic risk factors in Rwanda. We used the data from the 2015 Rwanda Demographic and Health Survey. We fitted two spatial logistic models with similar structures, only differentiated by the inclusion or exclusion of spatially structured random effects. The risk factors of stunting indicated by the geostatistical model were being male (OR = 1.32, 95% CI: 1.16, 1.47), birthweight (OR = 0.96, 95% CI: 0.95, 0.97), non-exclusive breastfeeding (OR = 1.24, 95% CI: 1.04, 1.45), diarrhoea in the last two weeks (OR = 1.18, 95% CI: 1.02, 1.37), being overweight for mothers (OR = 0.82, 95% CI: 0.71, 0.95), mother primary education (OR = 1.17, 95% CI: 1.03, 1.34). Also, poor flooring material in the household (AOR = 1.22, 95% CI: 1.06, 1.41) and non-improved water source (OR = 1.14, 95% CI: 1.01, 1.29, and wealth index groups were risk factors of stunting. Mapping of the spatial residuals effects showed that the Northern and Western regions followed by the Southern region of Rwanda still display high risk of stunting after accounting for all the covariates in the spatial model. Further studies are needed to examine the unknown spatially explicit factors that are correlated with the sustained high risk of stunting. Also, interventions to reduce stunting should be geographically targeted by taking into account stunting spatial heterogeneity and its underlying risk factors. Keywords: stunting, spatial pattern, Bayesian modelling, spatial residuals, Rwanda

5.1 Introduction

Stunting is still a significant public health issue in developing countries. Stunting is an indicator of chronic malnutrition and is manifested by low height-for-age in children less than five years. It is the most prevalent form of malnutrition in the world, with globally 146 million children under five stunted (UNICEF et al., 2019). The growth retardation starts during pregnancy and continues until a child is two years of age (Victora et al., 2010). Stunting develops from poor maternal health and nutrition, inadequate infant feeding practices and recurrent and subclinical infections (Weise, 2012). Stunting is a multifactorial and complex health problem (Huey & Mehta, 2016) and its direct causes are embedded into the complexity of household, societal and community factors (Stewart et al., 2013). The consequences of stunting are felt not only in childhood but also in adulthood. In children, stunting results in decreased motor and cognitive development, impaired immunity and low education attainment (Dewey & Begum, 2011). And in adulthood, it leads to lower economic productivity, increases the risk of chronic diseases and lowers offspring birthweight (Victora et al., 2008; Dewey & Begum, 2011).

Stunting reduction in children under five years of age is among the targets of the sustainable development goals 2 (UN, 2015). The aim of goal 2 is to end all forms of malnutrition by 2030, and by 2025 achieve a 40% reduction in the global prevalence of stunting as set by the World Health Assembly (Weise, 2012). On a global and regional scale, stunting prevalence varies spatially and temporally. The mapping of child growth failure in Africa (Osgood-Zimmerman et al., 2018a) showed that although stunting has reduced overall, there are persistent heterogeneities in levels and trends in stunting across the African continent. Also, targeting interventions to the most vulnerable has been mentioned as one of the main drawbacks in achieving desired targets in

stunting reduction. Osgood-Zimmerman et al. (2018a) estimated that if interventions and programmes to reduce stunting are not spatially targeted and monitored through taking into account the spatial heterogeneity in stunting, there will be likely no African countries to achieve the global nutrition targets in all its territories. The national prevalence can show improvement, while on a local level, the disparities in stunting levels remain high. The prevalence estimates used on national level mask the disparities and inequalities that exist within regions (Osgood-Zimmerman et al., 2018a). This is problematic because it is the aggregated prevalence levels that are usually used to direct locally implemented interventions and programs for stunting alleviation. Thus, in some instances, resources are most likely not well targeted to the most vulnerable. Also, most studies on the modelling of stunting and determination of its risk factors do not take into account the spatial dependency that exists in the data. Thus, to achieve the set target to reduce stunting, the spatial heterogeneity in stunting prevalence calls for more in-depth assessment of the drivers of stunting on a local level. Moreover, spatially targeted interventions and programs are required for the most vulnerable communities to benefit from them.

In Rwanda, stunting reduction has been made a priority, and this resulted in the national prevalence levels to be on the decline in past years. However, with a prevalence of 38% (NISR et al., 2015), the stunting levels in the country are considered high as per WHO thresholds (de Onis et al., 2019). Also, the disparity in stunting levels on a sub-national level is very apparent, with levels that vary from 59% in some districts to 17% (NISR et al., 2015). This heterogeneity might be even well pronounced at the local level. Thus, in this study, we sought to apply Bayesian geostatistical methods to study the spatial pattern of stunting in Rwanda by taking into account the spatial autocorrelation in stunting outcome in children less than five years. Bayesian geostatistics

offers a significant advantage over the classic statistical modelling of stunting. First, the spatial autocorrelation in stunting prevalence is taken into account, which allows for robust modelling of stunting as an outcome and its risk factors. Second, prior knowledge about the data, such as its distribution or extent of autocorrelation is taken into account. Third, the Bayesian models are robust to non-linear relationships that might exist between the outcome and the predicting factors. Fourth, the unexplained risk in stunting can be modelled and quantified through predicting the spatial residuals. Previous geostatistical analyses done on stunting in Rwanda focused on predicting stunting on a national level using geospatial covariates that are correlated with stunting (Spatial Data Repository & DHS Program, 2015; Osgood-Zimmerman et al., 2018a), but did not include child-related factors. Not including child-related covariates in the specified geostatistical model was highlighted as a limitation to shed light on pixel level heterogeneity in stunting determinants in these studies. In our current study, we aimed at specifying a Bayesian geostatistical model using child-related covariates to model stunting and study its risk factors in Rwanda. Also, we aimed to predict the spatial residuals effects to quantify the burden of stunting not accounted for by the specified geostatistical model. The findings of this study are expected to contribute in understanding the spatial heterogeneity of stunting in Rwanda, the risk factors that underline it and will provide new insights into the persistent high levels of stunting in some regions.

5.2 Methods

Study area

Rwanda is located in East African between 1° 04′ and 2° 51′ latitude South, and 28° 50′ and 30° 50′ longitude East. The country surface area is 26.338 Km^2 and is bordered by Uganda in the North, Tanzania in the East, Congo in the West and Burundi in the South. The country topography is made up of hills and valleys with the highest point in the

country being at 4500 m and the lowest at 980 m. The country is divided into 30 administrative units called districts. The stunting pattern in the country varies spatially with the Western province, located on the Congo Nile trail, being among the most affected, while the central region including Kigali has the least stunting prevalence (figure 18).

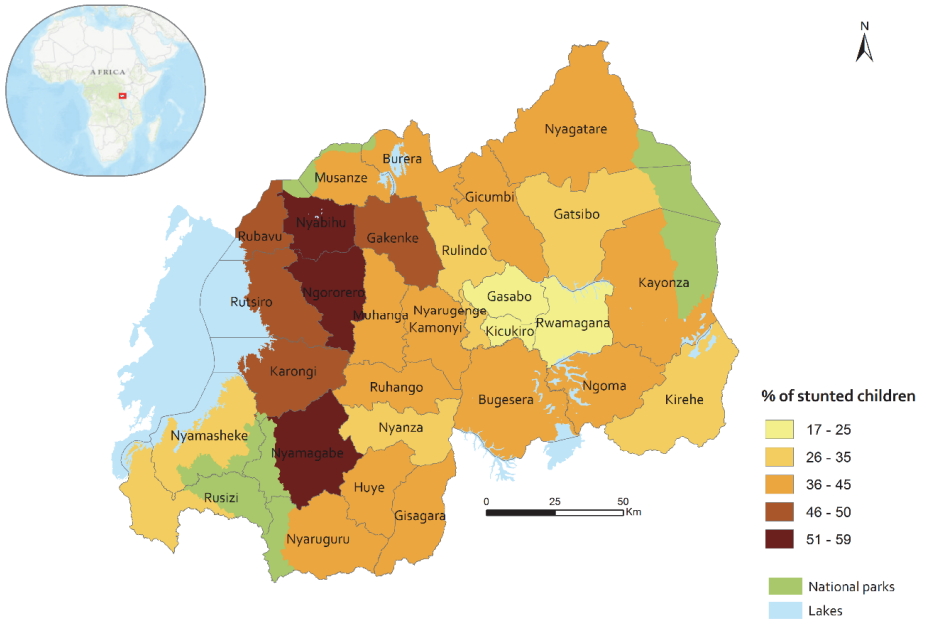


Figure 18. Stunting prevalence per district in Rwanda in 2015 (Source: DHS, 2015)

Data description

This study was based on the data from the 2015 Rwanda Demographic and Health Survey (NISR et al., 2015). In the first stage, clusters within enumeration areas that represent the 30 districts of Rwanda were selected. Clusters are the primary sampling units, and they contain on average 100 and 300 households from which 20 to 30 households are randomly selected for survey participation. In Rwanda, a total of 492 clusters were selected, 113 were located in urban areas, and 379 were located in rural areas. The second stage involved the systematic sampling of 26 households within each cluster, making up a sample of 12,792 households on a national level. From a sub-sample consisting of

50% of the households, indices of anthropometric status (child height and weight), individual mother and household characteristics were recorded for 3,813 children less than five years. The sample size of children less than five years with valid anthropometric data was 3593, and this study is based on this sample.

Child-related factors

The DHS data used in this study consisted of anthropometric, socioeconomic and demographic data of children less than five years. The dependent variable was the stunting status of children recorded as a dichotomous variable. The explanatory variables were age of child, birthweight, gender, preceding birth interval, exclusive breastfeeding in the previous six months, use of drugs for intestinal parasites, mothers' highest education level, and mothers' BMI. Exclusive breastfeeding and minimum dietary diversity were only measured for children less than two years (NISR et al., 2015). The socio-economic variables included the source of drinking water in the household, type of sanitation facility, type of cooking fuels, wealth index, and type of residence. These variables were considered because of their known causal effect on stunting (Stewart et al., 2013; Vilcins et al., 2018).

Statistical analysis

Descriptive statistics were conducted in SPSS version 24. Stunting was coded as 0 for non-stunting and 1 for stunting. For the categorical explanatory variables, the value with the least risk of stunting was set as the reference value (Sperandei, 2014). Correlation between variables was run to check for multicollinearity with $r > 0.7$. By taking into account the complex sample design of the DHS survey data, crosstab analysis was conducted on categorical variables with stunting as the dependent variable. For the continuous variables, univariate logistic regression was run to study the association of the variables with stunting. Unadjusted odds ratios were reported for both categorical and continuous variables and p-value of < 0.05 indicated statistical significance.

For spatial analysis, we conducted the analysis on an individual level by taking into account the spatial location of household clusters. We fitted a binary logistic model to the Bernoulli outcomes y_i, defining $y_i = 1$ if the child is stunted and $y_i = 0$ if the child is non-stunted. The logistic regression model with an expected probability of becoming stunted being *p* is thus,

$$Yi \sim Bern\,(pi)$$

$$\text{logit}\,(pi) = \beta_0 + \sum_{j=1}^{p} \beta_j X_{ij} + \sum_{k=1}^{k} \gamma_k Z_{ik} + s_i + u_i$$

where β_0 is the intercept, $\beta_1 \ldots, \beta_p$ and $\gamma_1 \ldots, \gamma_k$ are the unknown regression coefficients; X_{ij} is a set of metrical covariates, and Z_{ik} is a set of categorical covariates; s_i and u_i are random effects that allow for spatially structured variation (spatial random effect) and unstructured heterogeneity (non-spatial random effect), respectively.

For the spatial model inference, a Bayesian approach was used for estimating the posterior distribution of model parameters. Missing values in the covariates were addressed by using a covariate imputation model (Mason et al., 2012). For the continuous covariates, a normal distribution was specified $X_i \sim N\,(\mu_{Xi}, \sigma_X^2)$ with vague priors were assumed for the mean μ_X and variance σ_X^2. For the categorical covariates, we assumed a multinomial distribution $Z_i \sim MN(1, \pi_{ik})$, where π_{ik} are the probabilities for the $k = 1, .., K$ categories. This is results to $K - 1$ logistic regression models $\pi_{ik} = \exp(\eta_{Zik}) / \sum \exp(\eta_{Zik})$, $k = 2, \ldots, K$. We assumed $\eta_{Zik} \sim N\,(\mu_{Zk}, \sigma_Z^2)$ with vague priors for the mean μ_{Zk} and variance σ_Z^2. To complete the model, we assigned prior distributions for all unknown parameters. For the parameters of fixed continuous covariates, we assigned the normal priors $\beta_j \sim N(0, \sigma_\beta^2)$ with uniform standard deviations $\sigma_\beta \sim U(0,10)$. For the categorical covariates, we set $\gamma_1 = 0$ as

corner constraints for the reference categories and assigned the normal priors $\gamma_j \sim N(0, \sigma_\gamma^2)$, $j = 2, \ldots, J$, with uniform standard deviations $\sigma_\gamma \sim U(0,10)$. We modelled the spatially structured variation as zero-centered Gaussian random field with the multivariate normal (MV) distribution $s_i \sim MV(0, \Sigma)$ defined by the isotropic exponential correlation function $\Sigma_{ij} = \sigma_s^2 \exp(-\phi d_{ij})$. We assigned uniform standard deviation for the spatial variance parameter $\sigma_s \sim U(0,10)$ and the distance decay parameter (range) $\phi \sim U(0,1)$. We modelled the unstructured heterogeneity as normal exchangeable random intercepts $u_i \sim N(0, \sigma_u^2)$ with uniform prior standard deviation $\sigma_u \sim U(0,10)$. Regarding the covariate imputation models for the continuous covariates, the assumed vague normal priors are $\mu_X \sim N(0,0.001)$ and uniform standard deviations for $\sigma_X \sim U(0,10)$. Similarly, we assumed vague normal priors for $\mu_{Zk} \sim N(0,0.001)$ and the uniform standard deviations for $\sigma_Z \sim U(0,10)$.

We fitted two separate models with similar structures, except the inclusion or exclusion of spatially structured random effects. Model 1 included only the spatially unstructured random effects, while model 2 included both the spatially structured and unstructured random effects. The models were implemented in WinBUGS (Lunn et al., 2000), using Markov Chain Monte Carlo (MCMC) simulation techniques. We run a chain of 40000 iterations and discarded the first 20000, obtaining a final sample of 20000 for inference and summary statistics. Convergence was checked by visual inspection of the MCMC chains.

Model validation and comparison

We used the cross-validatory posterior Bayesian probability values (B *p-values*) and conditional predictive ordinates (CPO) to evaluate the predictive performance of the models. The CPO expresses the posterior probability of observing the outcome y_i when the model is fitted to all data except y_i. Larger values imply a better fit of the model to y_i, and

very low CPO values suggest that $y_i$is an outlier with regard to the model being fitted(McNeil & Wendin, 2007). The computation of B *p-values* involves sampling replicates from the models $y_i^{rep} \sim Bern(p_i)$ and estimating the probabilities $\Pr(y_i^{rep} = y_i|y)$. The CPO is the equivalence of leave-one-out as cross-validation. We calculated CPO for each observation as $\Pr(y_i|y_{[i]})$, where $y_{[i]}$ represents all data sets except y_i. Observations with high B *p-values* values are also indications of a good fit, while those with low values indicate a poor fit. For the comparison of models 1 and 2, we used the pseudo-marginal likelihood (PsML) measure, also a pseudo-Bayes factor. This is estimated as the product of the CPOs or the sum of their logged values (Gelfand, 1996). A higher value of the PsML implies a better fit of a model to the observations.

Prediction of spatial residual effects

The spatial residual effects of stunting were predicted on a 5x5 Km grid for the whole surface of Rwanda. The 5x5 Km grid surface was used to match previous published work on stunting prediction in Rwanda (Spatial Data Repository & DHS Program, 2015; Osgood-Zimmerman et al., 2018a). The use of 5x5 Km grid takes into account the displacement of household clusters, conducted by the DHS for respondent privacy protection (Burgert et al., 2013b). The spatial residuals were reported as odds ratios. Also, to understand the factors that could explain the predicted risk of stunting not accounted for by the geostatistical model, a visual comparison of the spatial pattern of the residuals was done with the results of a previous study by the same authors on the spatial pattern of stunting in Rwanda (Uwiringiyimana, Amer, et al.).

5.3 Results

Study population

The descriptive characteristics of the study population are presented in Table 25. Stunted children were older (31 months) than non-stunted children (27 months). The child birthweight was 3.4 kg for non-stunted children and 3.3 kg for stunted children. The preceding birth interval was two months higher for non-stunted children than for stunted children. Among the population, male children were 49.2% and female children were 50.8%; male children were more stunted (57.1%) as compared to female children (42.9%). Exclusive breastfeeding was low in this study population, only 25.2% of children were exclusively breastfed in six months. Only 12.9% of children had diarrhoea in the last two weeks, and within the stunting groups, 15.4% were stunted and 11.3% were not stunted. For 73.7% of children had received drugs for intestinal parasites in two weeks that preceded the survey. Children who had minimum dietary diversity were only 21.5%. Mothers who had secondary education were 12.9% and those who did not have an education were 14.4%; and most of the mothers (72.6%) had a healthy body mass index. For household factors, 30% of households had non-improved sanitation, 78.4% had poor flooring, 84.8% used poor cooking fuels and 28.4% had a non-improved source of drinking water. For the wealth index, nearly half of all the households were poor, with 24.8% under the poorest category and 22.0% under the poorer category. Most of the households (83.6%) were located in the rural area, with only 16.4% located in the urban area. Apart from the minimum dietary diversity factor, all other factors were significantly different between non-stunted children and stunted children.

Table 25. Descriptive characteristics of the study population (n=3593)

Covariates		Non-stunted (%)	Stunted (%)	N (%)	UOR*
Age (months)[a]		27 (0.4)	31 (0.4)	3593	-
Birthweight (kg)[a]		3.4 (0.0)	3.3 (0.0)	3328	-
Preceding birth interval (months)[a]		45 (0.7)	43 (0.7)	2586	-
Sex of child	Female	53.1	42.9	1768 (49.2)	1.00
	Male	46.9	57.1	1825 (50.8)	1.51
Exclusive breastfeeding	Yes	31.1	11.3	349 (25.2)	1.00
	No	68.9	88.7	1037 (74.8)	3.55
Diarrhoea in the last two weeks	No	11.3	15.4	462 (12.9)	1.00
	Yes	88.7	84.6	3131 (87.1)	1.42
Drug for intestinal parasite (in the last two weeks)	Yes	68.6	82.0	2646 (73.7)	1.00
	No	31.4	18.0	943 (26.3)	0.48
Minimum dietary diversity	Yes	21.7	21.2	316 (21.5)	1.00
	No	78.3	78.8	1152 (78.5)	1.03
Mother highest education	Secondary & higher	16.7	6.6	463 (12.9)	-
	Primary	71.1	75.4	2615 (72.8)	
	No education	12.1	18.0	516 (14.4)	
Mother BMI	Underweight	4.0	6.1	154 (4.8)	-
	Normal	70.0	76.6	2323 (72.6)	
	Overweight & Obese	26.0	17.3	724 (22.6)	
Sanitation	Improved	73.0	65.3	2494 (70.0)	1.00
	Non-improved	27.0	34.7	1067 (30.0)	1.43
Flooring	Good	27.1	12.6	769 (21.6)	1.00
	Poor	72.9	87.4	2796 (78.4)	2.58
Cooking fuels	Good/medium	18.9	9.2	540 (15.2)	1.00
	Poor	81.1	90.8	3023 (84.8)	2.30
Drinking water source	Improved	74.3	67.1	2551 (71.6)	1.00
	Non-improved	25.7	32.9	1014 (28.4)	1.42
Wealth index groups	Richest	21.3	9.1	599 (16.7)	-
	Richer	19.4	13.2	612 (17.0)	
	Middle	19.6	19.3	700 (19.5)	
	Poorer	19.1	26.8	791 (22.0)	
	Poorest	20.6	31.6	891 (24.8)	
Type of residence	Urban	20.2	10.3	591 (16.4)	1.00
	Rural	79.8	89.7	3003 (83.6)	2.20

[a]Means and standard errors are shown for continuous variables. *Measure of association (UOR: unadjusted odds ratio) was computed for only 2-by-2 tables. All variables were significant at p-value<0.001, except for minimum dietary diversity (p-value=0.840).

Figure 19 indicates the stunting prevalence at the household cluster level in children in Rwanda. At the cluster level, the prevalence varies from 0 to 100%. With the heterogeneity in stunting across the country, the high prevalence was predominantly found in the Western and Southern parts of the country. Kigali province has more household clusters with zero prevalence of stunting.

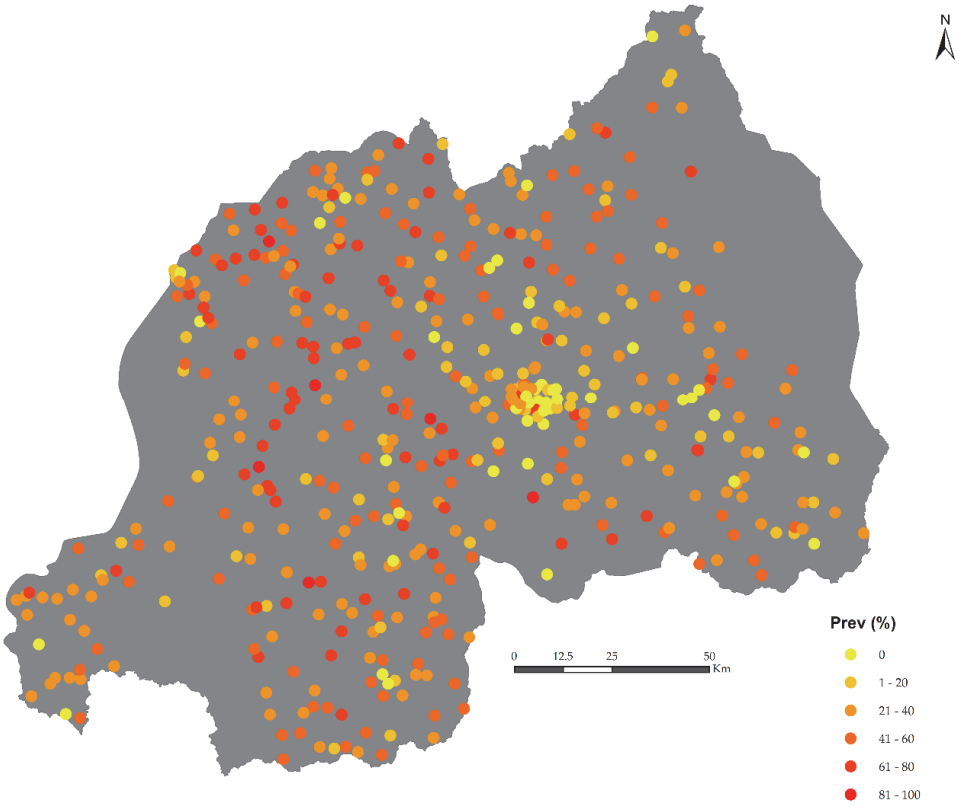


Figure 19. Stunting prevalence at household cluster level in Rwanda (source: DHS, 2015)

Model fit and comparison

The B p-values and CPO values ranged from 0.12 to 0.96 and 0.11 to 0.95 for model 1 respectively. For model 2, these values ranged from 0.12 to 0.96 and 0.11 to 0.95 for model 2 for the B p-values and CPO, respectively. These suggest a good fit for both models since no extreme low values were observed. Dividing either by their maximum to scale to

a maximum of one, the lowest values were approximately 0.12 for both models, far greater than a limit of 0.001 suggested by (Weiss, 1996). The PsML for model 1 was -2050.1, and that of model 2 was -2035.7, suggesting model 2 is superior to model 1.

Risk factors of stunting

The posterior means odds ratios and the 95% credible confidence intervals for the odds ratio of the Bayesian spatial model are shown in table 26. The geostatistical model indicated that age of a child, child's sex, birthweight, exclusive breastfeeding, diarrhoea in the last two weeks, BMI of mothers, mother education were significantly associated with stunting. Also, socio-economic covariates such as the type of flooring material in the household, type of water source, and wealth index group predicted significantly stunting.

We found out that the age of children was positively associated with the risk of stunting. On the other hand, the birthweight was associated with less risk of stunting in children (OR = 0.96, 95% CI: 0.95, 0.97). Male children had an increased risk of stunting compared to female children (OR = 1.32, 95% CI: 1.16, 1.47). Exclusive breastfeeding was protective against stunting; the odds of stunting were 24% higher in children who were not exclusively breastfed than in children who were exclusively breastfed (OR = 1.24, 95% CI: 1.04, 1.45). Similarly, the odds of stunting among children who had diarrhoea in the last two weeks that preceded the survey was higher than those who did not have diarrhoea (OR = 1.18, 95% CI: 1.02, 1.37). The odds of stunting were 15% higher among children whose mothers had primary education compared to children whose mothers had secondary and higher education level (OR = 1.17, 95% CI: 1.03, 1.34).

For socio-economic covariates, poor flooring in households significantly increased the risk of stunting in children (AOR = 1.22, 95% CI: 1.06, 1.41). For the wealth index variable, children whose household was in the

poorest category had increased risk of stunting (OR = 1.24, 95% CI: 1.07, 1.42). The use of non-improved water source for the household increased the risk of stunting by 14% compared to households that had improved source of water (OR = 1.14, 95% CI: 1.01, 1.29). The type of residence also was significantly associated with stunting, with households in the rural area having high risk of stunting compared to households in the urban area (OR = 1.17, 95% CI: 1.00, 1.36). Although some covariates such as the preceding birth interval, type of cooking fuels, and sanitation were not significantly associated with stunting in the spatial model, the direction of their relationship with stunting was correct. Besides, in the univariate analysis (Table 25) they were significantly associated with stunting.

Table 26. Risk factors for childhood stunting in Rwanda, 2015 from the binary logistic Bayesian geostatistical model

Covariates		AOR	95% CI
Age (months)*		1.01	(1.01, 1.02)
Birthweight (kg)*		0.96	(0.95, 0.97)
Preceding birth interval (months)*		1.00	(0.99, 1.00)
Sex of child	Female	1.00	
	Male	1.32	(1.16, 1.47)
Exclusive breastfeeding	Yes	1.00	
	No	1.24	(1.04, 1.45)
Diarrhoea in the last two weeks	No	1.00	
	Yes	1.18	(1.02, 1.37)
Drug for intestinal parasite (in the last 2 weeks)	Yes	1.00	
	No	0.76	(0.67, 0.87)
Minimum dietary diversity	Yes	1.00	
	No	0.93	(0.79, 1.08)
Mother highest education	Secondary & higher	1.00	
	Primary	1.14	(0.99, 1.31)
	No education	1.15	(0.99, 1.36)
Mother BMI	Underweight	1.00	
	Normal	1.01	(0.88, 1.15)
	Overweight & Obese	0.82	(0.71, 0.95)
Sanitation	Improved	1.00	
	Non-improved	1.06	(0.94, 1.21)
Flooring	Good	1.00	
	Poor	1.22	(1.06, 1.41)
Cooking fuels	Good/medium	1.00	
	Poor	1.06	(0.88, 1.24)
Drinking Water source	Improved	1.00	
	Non-improved	1.13	(1.00, 1.27))
Wealth index groups	Richest	1.00	
	Richer	0.89	(0.76, 1.03)
	Middle	1.01	(0.87, 1.17)
	Poorer	1.15	(0.99, 1.33)
	Poorest	1.24	(1.07, 1.42)
Type of residence	Urban	1.00	
	Rural	1.14	(0.99, 1.35)

* The continuous variables were centred around the mean. AOR: adjusted odds ratios

Spatial residual effects prediction

Figure 20 shows the spatial residual effects for stunting after accounting for covariates in the binary logistic spatial model, and figure 21 displays the corresponding prediction uncertainty. In figure 20, areas with higher odds ratios have increased risk of stunting, and areas with low odd ratios have decreased risk of stunting in children less than five years, after accounting for the covariates in the geostatistical model. The central area of the country that includes Kigali province and the Eastern part showed an unexplained low risk of stunting, while the Western, Northern and Southern regions showed an unexplained high risk of stunting.

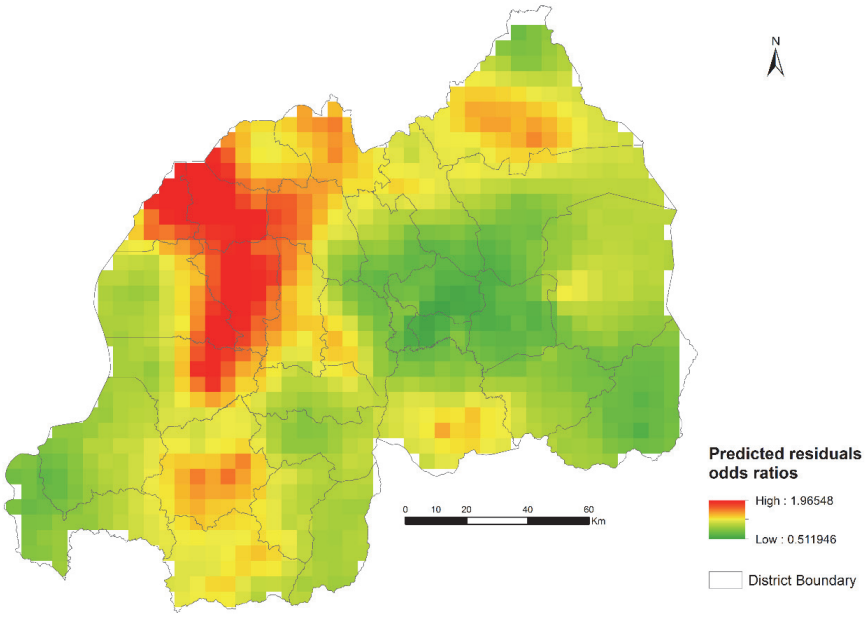


Figure 20. Spatial residual effects of stunting as odds ratios on a 5x5 Km pixel resolution based on geo-located household cluster-level data

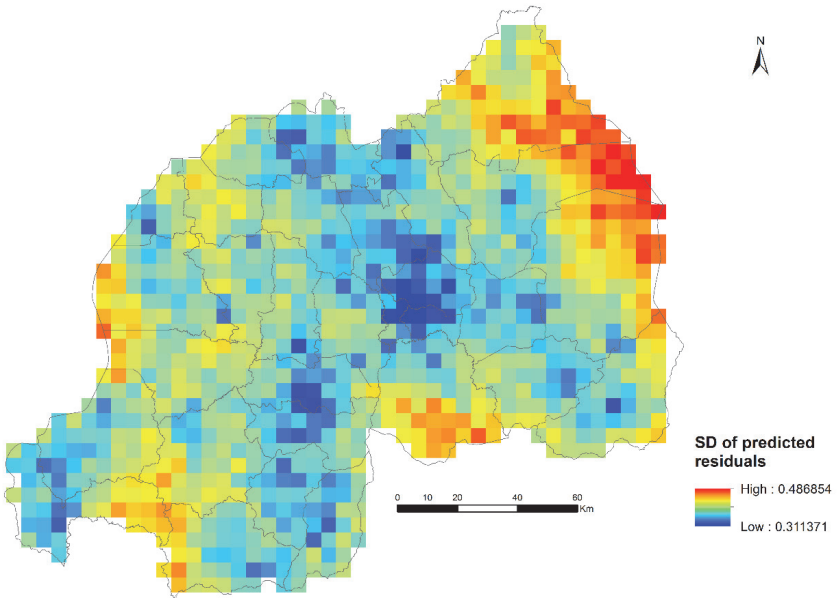


Figure 21. Uncertainty map for the posterior spatial residual effects displayed as standard deviation (SD) on 5 x 5 Km pixel resolution

5.4 Discussion

Our study provides evidence of the spatial variation of stunting in Rwanda and the risk of stunting that is still persistent after individual, maternal, socio-economic and dietary factors were taken into account. In the spatial model, child characteristics such as child age, child sex, birthweight, exclusive breastfeeding and having had diarrhoea in two weeks that preceded the survey were significant predictors of stunting. Previous research showed that male children are more likely to be stunted than female children (Kandala et al., 2011; Condo et al., 2015; Mukabutera et al., 2016). The odds of stunting increased as the age of children increased. However, the odds were not higher as previously shown in other studies (Kinyoki et al., 2015; Hagos et al., 2017), probably because age was considered as a continuous variable in the model. Consideration of age as a categorical variable would classify the risk of

stunting per age groups, and this risk has been shown to be higher in older children than in younger children (Dewey & Huffman, 2009; Uwiringiyimana et al., 2018b). The increase in birthweight of children was associated with slightly lower odds of stunting. As this association was significant, it highlights the importance of a child's weight at birth. Children who are born with a low birth weight (< 2.5 kg) are at a higher risk of stunting in the first 1000 days of their life (Amira M. Khan et al., 2017). In this study, non-exclusive breastfeeding in the previous six months was associated with the second highest odds of stunting after child's sex. The protective effect of exclusive breastfeeding on child stunting is a well-established evidence (Black et al., 2008; Stewart et al., 2013). Thus, the need for mothers to exclusively breastfeed their children within the first six months of life cannot be overemphasised. Children who had diarrhoea in the previous two weeks had increased odds of stunting compared to children who did not have diarrhoea. This result is consistent with the available evidence of the effect of diarrhoea on the linear growth of children (Checkley, Buckley, Gilman, Assis, Guerrant, Morris, Mølbak, et al., 2008). Early childhood enteric infections have been shown to lead to malnutrition, which in turn leads to increased vulnerability to infections (Guerrant et al., 2008; Guerrant et al., 2012). Micronutrients such as vitamin A and zinc deficiencies have been linked to the disruption in the intestinal barrier and absorption functions (Guerrant et al., 2008). In Rwanda, although most younger children are supplemented with vitamin A every six months (NISR et al., 2015), the dietary intake of these crucial micronutrients is still below nutrient requirements especially for dietary zinc intake (Umugwaneza et al., 2017; Uwiringiyimana et al., 2018b). Thus, to break this vicious cycle, proper sanitation measures on a household as well as community level, coupled with balanced nutrition especially in impoverished regions, are still needed. Unsurprisingly, households with access to a non-improved water source had high odds of stunting compared to households with access to an improved water source. The same pattern was observed for

households with poor flooring and households located in the rural area. These results confirm the impact the environmental and geographical milieu of a child has on increasing or reducing the risk of stunting. And this evidence is shown by the consistently high levels of stunting found in children living in rural areas as compared to their counterparts living in urban areas (Kandala et al., 2011; NISR et al., 2015).

The maternal factors considered in the model, mother BMI and education level, were both significantly associated with stunting. Children of overweight mothers had less risk of becoming stunted compared to children from underweight mothers. Also, having a mother with primary education increased the risk of stunting compared to having a mother secondary and higher education. The predicted association of mother's BMI and education with stunting is similar to the conclusion from previous studies (Wamani et al., 2007; Black et al., 2013; Yang et al., 2018), and emphasizes the importance of mother's health and knowledge on the nutritional wellbeing of her children. Although children of overweight and obese women were at low risk of stunting in this study, maternal obesity has been shown to increase the risk of childhood obesity, which can continue into adolescence and adulthood (Black et al., 2013). Children whose household was in the poorest category of the wealth index had the most odds of stunting compared to children from households in the richest category. The poorest category was the only statistically significant category, compared to poorer, middle and richer categories. Grantham-McGregor et al. reported similar positive association where living in absolute poverty was associated with poor development in children.

The spatial heterogeneity of predicted residuals observed in Rwanda (figure 3) suggests that unobserved factors not accounted for by the covariates in the geostatistical model contribute to the geographical disparities in stunting outcomes. The predicted spatial residual effects

depict a spatial pattern of the risk of stunting, which is pronounced mainly in the Western region, followed by the Northern and Southern regions of Rwanda. As stunting is multifactorial, factors contributing to stunting require further investigation. Although our model included not only child-related factors but also dietary covariates, other child and non-child related factors might be contributing to the unexplained variance. We believe among the factors non-accounted for in the model, the mycotoxins exposure in children is paramount (Smith et al., 2012). This is because there is strong and well-established evidence of the harmful effect of mycotoxins exposure on growing children. Evidence has shown that mycotoxins exposure, especially during the complementary feeding period, is associated with stunting in children (Gong et al., 2003; Okoth & Ohingo, 2004; Gong et al., 2004; Khlangwiset et al., 2011; Smith et al., 2012; Mupunga et al., 2017). The lack of mycotoxins data on a national level in Rwanda has been highlighted by the authors before as a challenge in analysing this effect (Uwiringiyimana, Amer, et al.). In the first exercise of stunting the spatial pattern of stunting in Rwanda, a proxy measure of aflatoxins exposure was used (Uwiringiyimana, Amer, et al.).

Consequently, in this study, an attempt is made to use a similar proxy variable of mycotoxins exposure to spatially visualise and inspect if there is any relationship with the unexplained variance in stunting. The proxy variable for mycotoxins used by Uwiringiyimana, Amer, et al. classified household clusters as being at high or low risk of mycotoxins exposure depending on whether clusters were served by an urban market, a rural market or neither served by an urban nor a rural market. An urban market was considered to be at the start of the food supply chain, while a rural market was considered to be at the low end of the food supply chain of complementary flours. Consequently, clusters that were served by an urban market were considered to have a lower risk of exposure to contaminated complementary flours that are supplied

through the food supply chain (Uwiringiyimana, Amer, et al.). The household clusters not served by an urban nor a rural market were considered to have the highest risk of exposure to contaminated flours.

Figure 22 shows the spatial residual effects map overlaid with the spatial distribution of household clusters that are served by either an urban market, a rural market only, and neither an urban nor a rural market within a 5 Km (A) and 10 Km (B) radius of the household cluster. For both maps, especially panel B, the green zones representing a lower risk of stunting are associated with household clusters that are served by urban markets. According to our hypothesis, these household clusters have a low risk of exposure to mycotoxins. This observation implies that household clusters served by an urban market most likely have access to better quality of foods in relation to mycotoxins exposure. The correlation between the residual odds ratios, sampled at each household cluster location, and the three classes of household clusters was significant for both panel A ($r=0.232$, p-value<0.01) and panel B ($r=0.279$, p-value<0.01). Also, a pattern is observed in the Western and Southern provinces where the high unexplained risk for stunting coincides with the household clusters that are not served by neither an urban or a rural market (figure 22 A), and household clusters that are served by mostly rural markets (figure 22 B). On the contrary, the Eastern region which also features clusters with no access to neither an urban nor a rural market (figure 22 A), still depict the lower risk of stunting. Because we believe there is an interplay of factors both in the regions with high risk of stunting or regions with low risk of stunting, children in the high risk regions might be already at a disadvantage, in such a way that the addition of mycotoxins contamination drastically affect them compared to children whose only risk would be the exposure to mycotoxins. We highlight that this comparison takes into account the limitations of using the proxy variable of exposure to mycotoxins as discussed in Uwiringiyimana, Amer, et al.

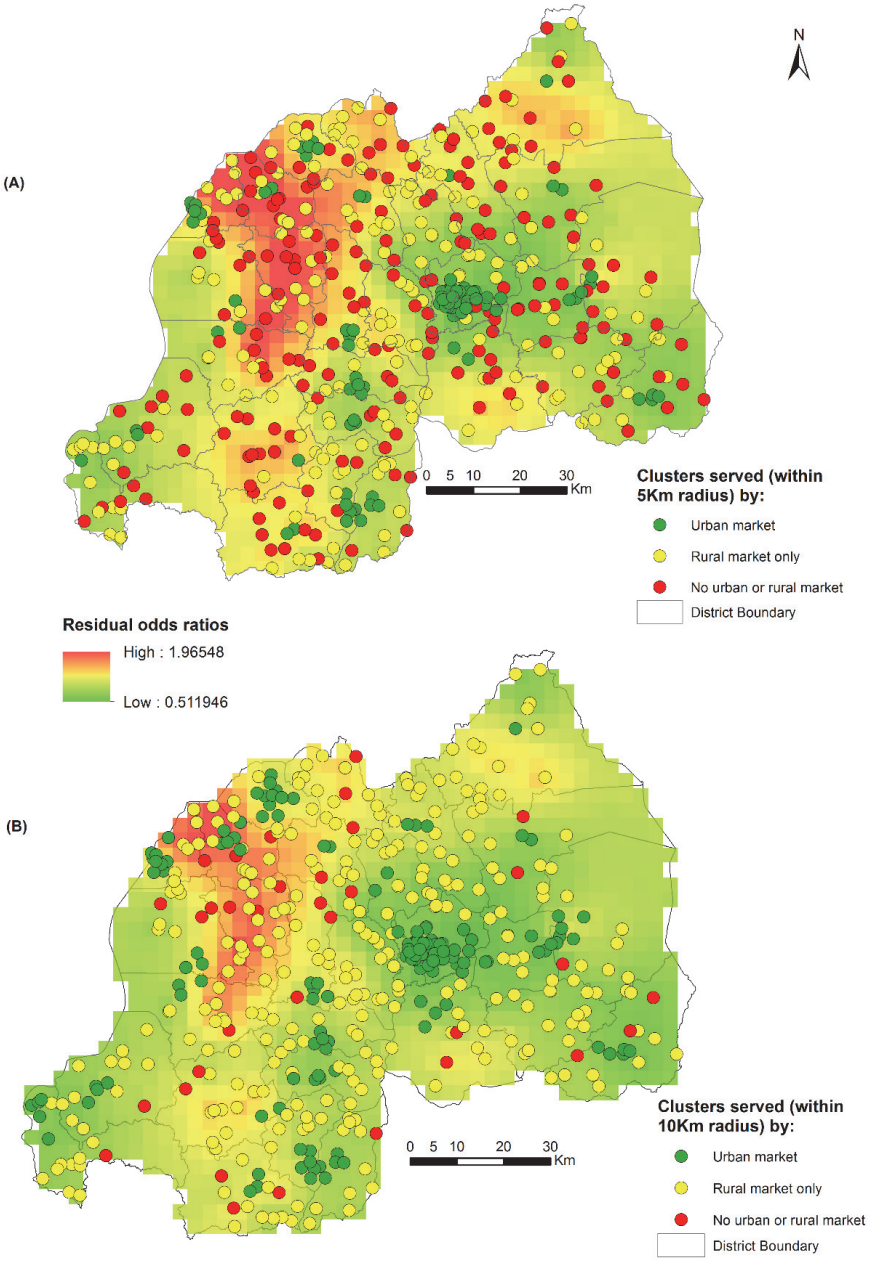


Figure 22. Spatial residual effects map overlaid with the spatial distribution of household clusters that are served by either an urban market, a rural market only, and neither an urban nor a rural market within a 5 Km (A) and 10 Km (B) radius of the household cluster.

Elevation is another factor that could explain the residual variability in stunting as it is associated with stunting in children (Dang et al., 2004; Balk et al., 2005). Although not considered in our model, the regions on figure 3 with the high unexplained risk of stunting coincide with regions also with high altitude in Rwanda. Previous research works spatially predicted stunting on a national level in Africa and Rwanda (Spatial Data Repository & DHS Program, 2015; Osgood-Zimmerman et al., 2018a). However, the prediction of stunting was done using non-child related covariates such as surface temperature, night time lights and elevation. Our study strength lies in using child-related factors that are known to have a causality relationship with stunting in the geostatistical model, to study the risk factors of stunting in Rwanda and predict the spatial residuals effects of stunting. Predicting the spatial residual effects by incorporating individual and household related factors has been done previously in other studies, to learn the spatial distribution of *Schistosoma mansoni* infection in Ivory Coast (Assare et al., 2015) and of malaria in Malawi (Kazembe et al., 2006). All the variables included in the spatial model, except for the minimum dietary diversity, were strong predictors of stunting in children. Although the association of the minimum dietary diversity with stunting was not significant in the univariate analysis, we kept the covariate in the spatial model because of its known effect on stunting in children (Krasevec et al., 2017). Applying Bayesian geostatistical modelling to our data had different advantages. First we could apply prior knowledge in the model using published sources (Spatial Data Repository & DHS Program, 2015). Second, using covariates strongly associated with stunting, the spatial residual effects of stunting that display the unexplained risk of stunting in Rwanda were predicted. Third, normal regression methods usually applied to study the risk factors of stunting do not take into account the spatial heterogeneity and spatial dependence that exist in stunting. Our model used the geographical locations of household clusters to estimate the spatial dependency in childhood stunting in Rwanda.

Our study had some limitations. First, because the covariates such as exclusive breastfeeding and minimum dietary diversity are measured by DHS only for children < 2 years, this introduces missing values in the data for all children aged >2 years. Consequently, the small sample of children with dietary data might have led to the lack of association between minimum dietary diversity with stunting. Second, although our study is the first to apply geostatistical modelling to predict unexplained risk of stunting in Rwanda, our model did not take into account environmental factors that influence stunting. Thus, further studies on modelling the unexplained risk of stunting can research on the underlying factors that determine the spatial pattern of the high and low odds of the spatial residuals effects observed in this study. The model can be strengthened by incorporating non-child related factors that could shed light on the unexplainable odds ratios of stunting. Also, for future research, the extent of the negative effect of mycotoxins exposure on the growing children urgently needs to be evaluated. Studies determining the extent of mycotoxins contamination along the food supply chain in Rwanda, by taking into account the spatial variation in mycotoxins, can provide the needed data to examine this relationship. The results would serve to inform policy on the extent of mycotoxins contamination in the food supply chain and provide spatially informed evidence of the effect of mycotoxins exposure in determining stunting spatial pattern in Rwanda.

5.5 Conclusion

Stunting in Rwanda varies spatially on a local scale, below the usual heterogeneity displayed on a district level. Our study applied advanced geostatistical method to study the risk factors of stunting in Rwanda and predict the unexplainable odds of stunting in the study population. Although stunting is multifactorial, to have a deeper understanding of its drivers on a local scale, studies that take into account stunting spatial dependency are warranted. Using Bayesian geostatistical modelling

offers this opportunity of studying the spatial pattern of stunting by taking into account its spatial dependency and by revealing residual stunting risk unexplained by the model. The areas with high spatial residual odds ratio imply that further studies are needed to look into unknown spatially explicit factors that are correlated with the sustained high risk of stunting.

Chapter 6 Synthesis

Rwanda has made progress in reducing the prevalence of acute malnutrition in children, however chronic malnutrition is still prevalent. Also, the prevalence of stunting is spatially variable across the country and the causes of the persistent high levels of stunting in some regions remain unknown. As described in Chapter 1, the quality and safety of children's complementary diet needed to be examined closely, especially because some regions, known to have enough food such the Northern region of Rwanda, show high levels of stunting. Thus, this thesis aimed to examine the determinants of stunting by focusing on the complementary feeding practices, diet quality, mycotoxins exposure and environmental factors. The first two chapters of this thesis focused on studying stunting on a local level through a case study conducted in the Northern region of Rwanda in the district of Musanze. The last two chapters focus on determining the pattern of stunting in Rwanda on a national level by taking a holistic approach in examining all the relevant factors known to influence stunting and most importantly examining the mycotoxin exposure factor that have not yet received much attention in the Rwandan context. Moreover, application of advanced spatial statistical techniques is the first ever to take a different and more advanced analysis of stunting. The local study was based on primary data collected in Musanze District while the national level analysis was based on secondary data, primarily the publicly available data from the 2015 Rwanda Demographic and Health Survey.

6.1 Summary of research findings

The regional study on complementary feeding practices of children in Musanze district in Rwanda as described in Chapter 2, shows that stunting is still very prevalent in the region. The main factors associated with height-for-age in children were age, exclusive breastfeeding, deworming tablets use in the previous six months, the caregiver BMI and dietary zinc intake. The logistic regression model showed that as age increased by one month, the risk of stunting in children increased by

20%. On the contrary, the odds of stunting reduced significantly when the child had received deworming tablets or had been exclusively breastfed. This study was among the few that assessed the dietary intake of children in Rwanda, including dietary zinc intake.

The aflatoxins contamination level in maize and peanut collected from household food samples was determined in Chapter 3 to study the effect of exposure to aflatoxins on stunting in the same population of children as in Chapter 2. The levels of aflatoxins in maize and peanut flours used for complementary feeding of children were very high, far exceeding the regulatory limit for aflatoxins in East-Africa for peanut flour. The exposure to aflatoxins was significantly and positively associated with stunting after adjusting for the previously identified predictors of stunting in Chapter 2. This result confirms our hypothesis that aflatoxins exposure in children in Rwanda is associated with stunting, and could thus, be one of the missing links to the persistent high levels of stunting in some regions.

In Chapter 4, a national level analysis was conducted to examine the stunting spatial pattern of stunting in Rwanda and study the determinants of stunting focusing on socio-economic and biophysical factors, and a proxy measure of mycotoxins exposure. Child and maternal factors associated with stunting were age, being male, birthweight, height of mothers and secondary education or higher. The examination of biophysical factors revealed that the elevation of a household cluster was negatively associated with height-for-age of children, implying the influence of remoteness on the health and nutrition of children. The elevation factor can influence accessibility to foods and health care services, which in turn influence child growth. Because of the lack of spatially detailed data on mycotoxins in Rwanda, a proxy measure of mycotoxins exposure was used by considering households clusters served by rural markets located at the end of the

food supply chain as having a higher risk of exposure to mycotoxins. This proxy measure was found to be negatively associated with height-for-age, for household clusters with access to a rural market within a 10 Km radius. Our results call for more research on the extent of mycotoxins contamination along the food supply chain in Rwanda and the impact of the exposure to aflatoxins on stunting.

Stunting on a national level was modelled using advanced geostatistical modelling in Chapter 5. A Bayesian logistic regression model was used to study the risk factors of stunting and predict the risk of stunting not accounted for by child-related covariates previously included in the model. From the spatial model, child factors significantly predicting stunting were child age, child sex, birthweight, exclusive breastfeeding and having had diarrhoea in two weeks that preceded the survey, mother BMI and mother education. In addition, wealth index, household flooring and access to improved water source were also associated with stunting. The prediction of spatial residual effects of stunting showed a spatially explicit pattern of unexplained high and low risk of stunting in some regions in Rwanda, especially the Western part. This result implies that unobserved factors not accounted for in the model contribute to stunting. The pattern of the spatial residuals corresponded to the proxy factor of exposure to mycotoxins and to elevation. Thus, further studies are needed to look into the unknown spatially explicit factors that determine the risk of stunting.

6.2 Reflection on research findings

This section reflects on the main findings of this research, by focusing on the two scale levels used namely the local studies conducted in the Northern region of Rwanda and the national level spatial analysis of stunting.

Regional study of stunting determinants

The case study in Musanze district gave evidence on the quality and safety of the complementary foods used to feed children. The diet of most households was mainly plant-based, with limited consumption of animal source foods. This implies that children were lacking important micronutrients from their diet such as zinc, especially because zinc from plant-based foods is less bioavailable due to the presence of phytates. Thus, a continued effort in the implementation of programs and interventions that focus on diet diversification by incorporating animal source foods is needed. The consumption of animal source foods especially small dried fish was also included in a food based dietary guideline for children in Rwanda (Umugwaneza et al., 2017). As part of our research, soil samples Musanze area were collected to examine the zinc soil content. The analysis was conducted because zinc deficiency in the soil affects the zinc level of crops, which in turn can lead to lower zinc content of the diet. Our results showed that there was no difference in zinc levels of the soil samples collected at different elevation levels, although there was a slight positive gradient in zinc levels with respect to increased elevation.

A major challenge in the dietary survey we conducted to assess the complementary feeding practices was the lack of a national food composition table. Rwanda does not have its own food composition table, and it required us to compile one for the purpose of this thesis. As mentioned in chapter 2, nutrient values of foods consumed by children had to be imported from food composition tables from neighbouring countries such as Uganda and Tanzania, and also from West Africa using the West-Africa food composition table. To properly make dietary recommendations and formulate guidelines for energy and nutrient intake, a country needs a food composition table. This is still a major drawback for a country as Rwanda that is thriving to alleviate the problem of chronic malnutrition in children. With a proper food

composition table, research can be conducted to identify nutritional problems and set nutrient intake desirable for health in vulnerable groups such as growing children, pregnant and lactating mothers (Greenfield & Southgate, 2003). And this data can in turn be used for food and nutrition policy formulations for setting up dietary interventions or food fortification programmes (Greenfield & Southgate, 2003). The importance of a food composition table is further heightened by the geographical variability in diets that exists within a country. Thus, dietary interventions in Rwanda need to be geographically targeted by considering the dietary disparities that exist within regions to make specific recommendations accordingly.

Musanze district in which our case study was conducted is particularly concerned. This is because it is the district known as the food basket of Rwanda, as it supplies most of the potatoes and cabbage consumed all over the country. On the other hand, it was the district with the highest level of stunting in 2012, with 6 out of 10 of every child less than 5 years stunted (WFP et al., 2012). Contrarily, the district was also among the districts in the country with less unacceptable household food consumption. In our research, we found that the diet of children was insufficiently diversified with little to no consumption of animal source foods. In addition, due to the climate in the region and the use of imported peanuts to prepare complementary dishes for children, we hypothesized that mycotoxins contamination of complementary flours could be a factor contribution to the high stunting levels. Thus, we conducted the next study (Chapter 3) where we quantified aflatoxins contamination of maize and peanut flours collected in households, estimated the exposure of children to aflatoxins, and finally studied its association with stunting. Our study was the first to quantify the mycotoxins from complementary flours collected from households. We found that the exposure to aflatoxins was associated with lower height-for-age in children. The quantification of aflatoxins in maize and peanut

flours and the positive association of exposure with stunting was an important step in this research. In Rwanda, no other study has been done before to elucidate the possible contribution of mycotoxins contamination of flours on the stunting levels in the country. On the other hand, the use of lateral flow immunoassay strips to quantify the extent of contamination in flours was suitable for our research. This method can be appropriate in poor-resource settings, where laboratory facilities with conventional analytical techniques is scarce and often lacking. The main challenge in our study was that not every household visited had maize and peanut flour to sample, which calls for a larger study to quantify the extent of contamination with aflatoxins in households in Rwanda.

Modelling of the determinants of stunting on national level

In the second part of this thesis, the determinants of stunting were studied on a national level using secondary socio-economic and environmental data from publicly available sources. The stunting pattern was first studied in Chapter 4, followed by a more advanced modelling of stunting done in Chapter 5. The study of the spatial pattern of stunting revealed the child-related and environmental factors that were associated with stunting in children less than two years in Rwanda. The analysis of stunting pattern was based on the household cluster locations, provided by the DHS. Using these clusters locations allowed for the mapping of stunting on high spatial resolution, displaying the granularity in stunting prevalence across the country. Mapping stunting on a cluster level contrasted with the usual reporting done on a district level. This latter masks the existing heterogeneity in stunting on a household cluster level (figure 17 in Chapter 4). Most policies implemented in Rwanda to alleviate stunting uses the prevalence on a district level as a guide to where programs and interventions should be implemented or reinforced. The implementation of programs can be improved by targeting communities and areas with highest prevalence levels within districts. In Chapter 4, we further

analysed the possible effect of mycotoxins exposure on height-for-age. We had previously planned to use the data by the Rwanda Agricultural Board (RAB) on aflatoxins contamination in maize (Boniface et al., 2016). RAB conducted the mycotoxins survey analysis in maize and cassava by collecting samples from different sources such as markets, farmers, fields and by classifying samples into pre-harvest and post-harvest samples. The data of our interest was the aflatoxins contamination levels in post-harvest maize flour. Figure 23 shows the location of the post-harvest maize samples and their sources. The RAB data was unsuitable for our analysis because of two main reasons: first, as shown in figure 20, the data on aflatoxins in maize samples collected at post-harvest was not spatially distributed enough because some districts were not sampled, and thus the data could not be representative for the whole country. Second, for the sampled districts, the sample number was not sufficient enough to represent the district for a district level study. Also, the maize samples were from different sources. In total 167 samples of post-harvest maize had been collected: 25 samples from farmers, 30 samples from the fields, 9 from markets, 3 from maize processors, 46 from stores and for 54 samples the sample source was missing.

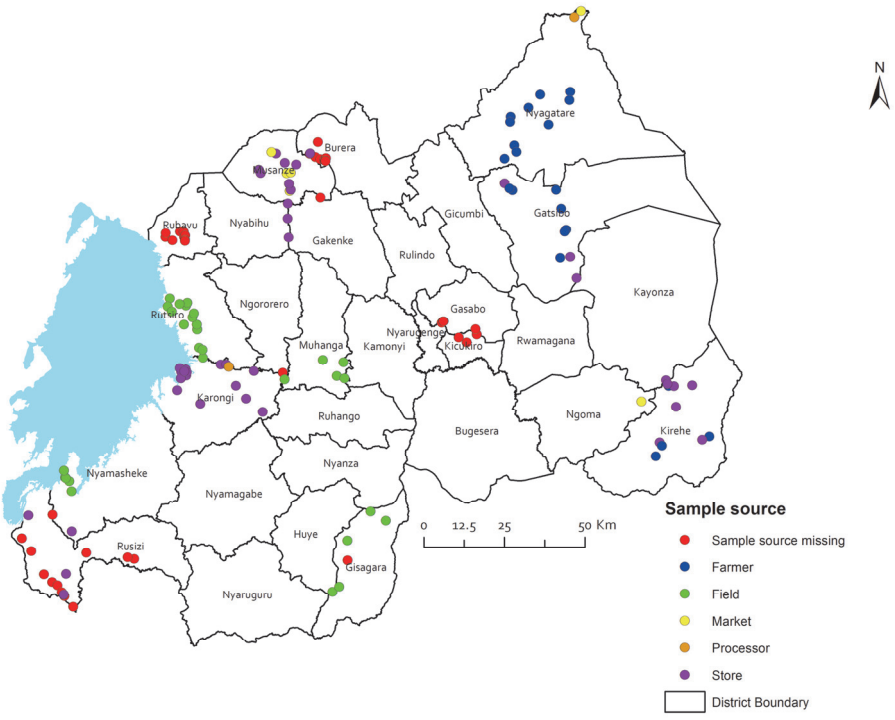


Figure 23. Location and sample source of post-harvest maize flour from the RAB mycotoxins survey per district

Due to this data constraint, we used the proxy measure of aflatoxins exposure. We consider household clusters that obtain flour from markets at the lower end of the food supply chain as being at risk of exposure to higher levels of aflatoxins from complementary flours compared to household clusters that obtain flour from markets at the start of the food supply chain. For this assumption, two distance parameters of 5 Km and 10 Km were used. The association between the distance variables with height-for-age was significant for clusters that were served by an urban market vs clusters that were served by a rural market within a 10 Km distance. Household clusters that were served by a rural market within a 10 Km radius had a negative association with height-for-age compared to household clusters that had access to an urban market. Given the hierarchy of markets in Rwanda, this results is meaningful and implies that market locations can have influence on the

length of storage of complementary flours. As with increased storage time, the risk of mycotoxins production increases, people served by markets at the lower end of the food supply are likely to have a higher exposure to aflatoxins. In Chapter 5, a geostatistical model was built to take into account known strong predictors of stunting, particularly dietary factors such as exclusive breastfeeding and minimum dietary diversity factors. In addition, our model was designed to predict the stunting spatial residuals. Predicting residuals quantified the risk of stunting that remains after the rest of the predictors were taken into account. The classic logistic regression only predicts the risk of stunting accounted for by every covariate in the model and spatial dependency in the outcome is not considered. Spatially predicting the risk of stunting unexplained by the model provides insights about the possible unknown or overlooked factors not accounted for in the model. For Rwanda, the spatial residuals displayed a clear spatial pattern; indicating that for regions such as the Western province with unexplained high risk of stunting, more factors other than the factors included in the model are contributing to the stunting levels observed. As the Western province remains the province with the highest levels of stunting in Rwanda, a more thorough examination of the stunting determinants in the region is required.

6.3 Potential to use routinely collected data on stunting

For the second part of this thesis on spatial modelling of stunting on a national level, we had initially planned to use the routinely collected data on stunting available from the Rwanda Health Management Information System (HMIS) (USAID/Rwanda, 2006). HMIS is a health system largely used in Rwanda by the Ministry of Health (MOH) and the Rwanda Biomedical Centre (RBC) for routine and systematic collection of health data at health facility level for different public health indicators such as malaria, parasitic disease rates, HIV infection, and

other indicators of public significance. HMIS provides health records per month and year from all individual health facilities in Rwanda, and this data is automatically added into the national database. The data in the HMIS system is highly spatially detailed as it links to each health facility location. This offers a great advantage for spatially explicit analysis of recorded public health indicators. Figure 24 shows the location of health facilities in Rwanda.

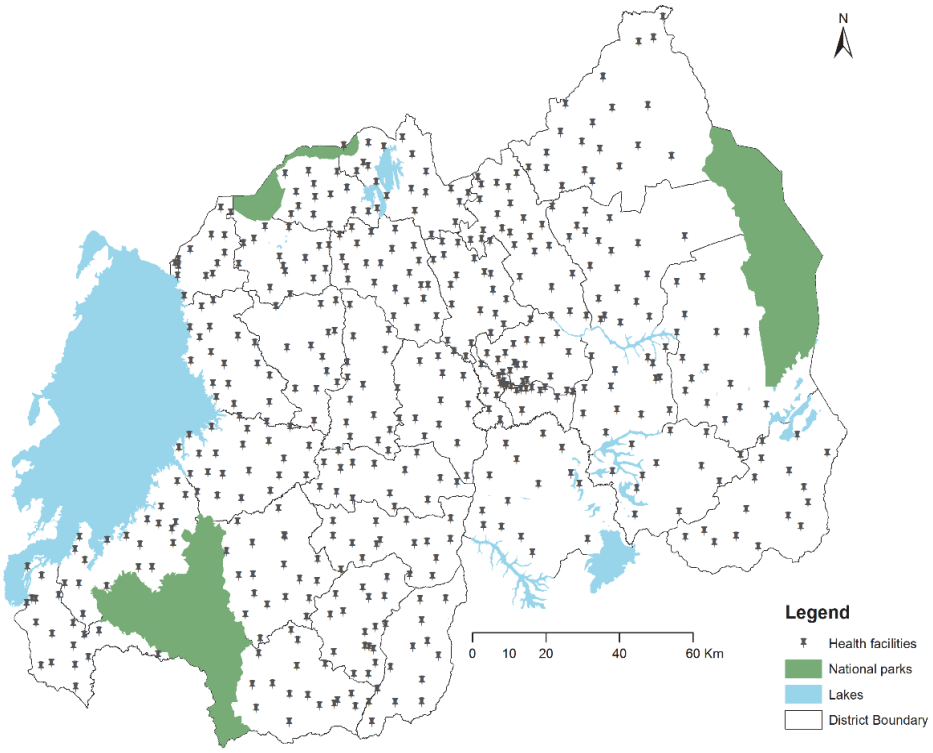


Figure 24. Health facility locations in Rwanda (Source: Rwanda Biomedical Center)

Since 2012, stunting cases data are collected from each health facility in Rwanda through HMIS. All children that are brought to a health centre for any treatment, are screened for stunting by recording their anthropometric measurements. All children found to be stunted or malnourished are transferred to the nutrition centre for follow-up and their anthropometric measurements are recorded into the HMIS system.

This data was available for per month for the year 2012 to 2017. After processing the data and running initial analyses, however, we realized that the data has several flaws. The major flaw was that the system does not differentiate the counts of stunted children recorded per health facility between the counts of children who were recorded before and new cases of stunting. In other words, the recording unit of the system is per consultations and not individuals. There is no specific track of the previously recorded children. Thus, the counts of stunting recorded each month per health facility mostly contained duplicate (or even more) records of every child measured. For example for the year 2015, the total number of screened children at health facilities was 4,350, 187 which far exceeded the total number of children under five in Rwanda which was around 1,676,914 in the same year. The total children population in Rwanda was estimated based on the 2012 Census data (NISR & MINECOFIN, 2012). Another flaw of HMIS data on stunting was that not every health facility was entering data in their system. Thus, some health facilities had zero counts of stunted children, which does not mean that there are no stunted children in the region covered by the health facility.

On the other hand, the routinely collected data of children offers great potential for monitoring stunting in Rwanda. This is because as there is currently no system to monitor stunting in children in Rwanda. The country waits for the Demographic and Health Survey that takes place every five years to know the prevalence levels of stunting and thereby take the necessary measures in order to eradicate stunting, especially in regions with high levels. Because HMIS is an already established system that provides very spatially detailed data on other health metrics, the correct recording of stunting cases for example by assigning an identification number to every child recorder in the system would offer great potential to monitor it all over the country. Consequently, timely measures or policies can be taken to address the high levels of stunting

especially where hotspots of stunting are persisting or (re-)appearing. The monitoring of stunting would serve not only for planning interventions to alleviate stunting but also for the prevention of stunting. Moreover, when the correct data is available it would spear ahead research on stunting in Rwanda.

In 2014 for example, in order to review the national nutritional policy that had been implemented in the previous five years, the government of Rwanda required to have an overview of the stunting prevalence. Thus, a nationwide screening of malnutrition in children was conducted by the College of Medicine and Health Sciences (CMHS) and included 1,277,670 children, representing 70% of all children less than five years in Rwanda at the time of the survey (Lyambabaje et al., 2015). An overview of the data sources of stunting prevalence used in Rwanda for the year 2015 with the incidence data provided by HMIS is given in table 28. Another survey that provides data on the prevalence of stunting is the Comprehensive Food Security and Vulnerability Analysis (CFSVA) survey which takes place before the DHS survey. We visualized the stunting prevalence data obtained through the four data sources, to check if the data was comparable. As shown in figure 25, generally there was some agreement between the prevalence data from DHS, CFSVA and CMHS, with the Western and Northern regions having high stunting levels. On the other hand, because of the nature of the data from HMIS, some regions with lowest stunting levels (<30%) had actually high levels when compared to the rest of the three maps. As shown in table 27, the correlation between the data from HMIS and the data from DHS, CFSVA and CMHS was very low. This low correlation confirms that the current HMIS data would not be reliable to be used for research. Besides, by comparing the DHS and the HMIS data, there are other inherent reasons why the HMIS data does not compare well with the DHS data. First, the DHS data is sample-based and is collected on household level and aggregated to district level. On the other hand, the

HMIS data is incidence data routinely collected from health records from individual health facilities. Second, because the incidence data comes from children who only visited the health facilities, there might a problem of representativeness or under-reporting: stunted children whose mothers do not bring them to the health facility are thus not accounted for.

Table 27. Correlation (r^2) between the four sources of stunting data aggregated on a district level

	DHS	HMIS	CFSVA	CMHS
DHS	1	0.02	0.62	0.42
HMIS		1	0.03	0.0024
CFSVA			1	0.36
CMHS				1

DHS: Demographic and Health Survey; HMIS: Health Management and Information System, CFSVA: Comprehensive Food Security and Vulnerability Analysis Survey; CHMS: College of Medicine and Health Science

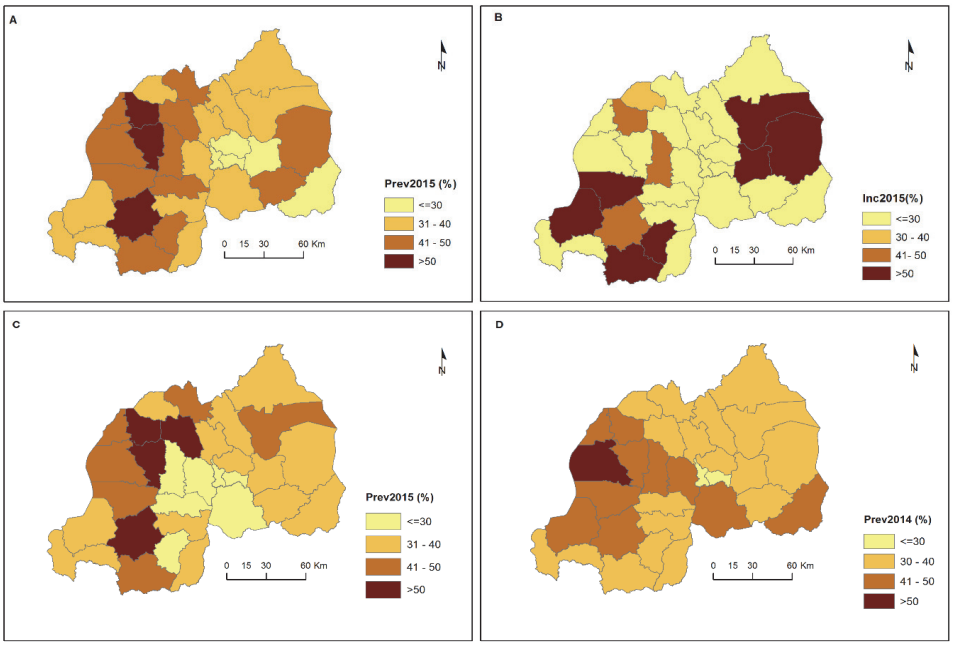


Figure 25. Stunting prevalence from DHS (A), stunting incidence from HMIS (B), and prevalence from CFSVA (C) and CMHS (D) per district in 2015

By mapping the data from the nutrition screening survey by the CMHS on a health facility service area level (Nyandwi, Veldkamp, Amer, et al., 2017) as shown in figure 26, an insightful spatial variability in stunting is obtained, that contrasts the usual reporting done on the district level. Rwanda has 376 health facility service areas delineated around each health facility. As shown on figure 26, some areas, in the South East for example, that would be considered as having low stunting due to mapping on a district level clearly show high levels of stunting that could otherwise be overlooked for policy or program targeting.

Table 28. Comparison of data sources for stunting prevalence in Rwanda in the year 2015

	DHS	CFSVA	CMHS	HMIS
Type of data	Prevalence	Prevalence	Prevalence	Incidence
Time (frame) of data collection	9 November 2014- 6 April 2015	April-May 2015	March-April 2014	Collected monthly in 2015
Sample design	1. Selection of 492 clusters (113 urban & 379 rural) within sample PAs which are the 30 Districts of Rwanda; 2. Selection of 26 HHs within each cluster; 3. 12792 HHs included (final sample was 12793 HHs, one HH being actually two HHs)	1. Selection of 25 villages per district; 2. Selection of 10 HHs in each village; 3. 7300 HHs included in the final sample was 12792 HHs included stage	All children less than 5 years in Rwanda were eligible. Those screened represented 69.3% of the Rwandan children population who are less than 5 years (as per the projected population by the end of 2013)	Data is collected from 543 HFs (includes HCs, DHs and NRHs) in Rwanda
Number of children surveyed for anthropometry	3,813 children less than five years in a subsample of 50% HHs	4,038 children less than five years (information on IYCF collected for 1379 children less than 2 years)	1,277,670 children less than five years	Total number of screening done: 4,350,187. Note: children were screened more than once throughout the year
Coverage/representativeness	National level using sample weights, representative data on both province and district level	National level using sample weights, representative data on both province and district level	National level	National level and Health facility level
Spatial unit of publicly available data	Household clusters	District	Health centres	Health centres
Government use of data	Official use of data to set policies and programs to fight stunting	Compliments the data from DHS	Used only for the year 2014	Currently not used

DHS: Demographic and Health Survey, CFSVA: Comprehensive Food Security and Vulnerability Analysis, CMHS: College of Medicine and Health Sciences, HHs: households, HFs: Health facilities, HCs: health centres, DHs: district hospitals, NRHs: National Reference Hospitals, IYCF: infant and young child feeding.

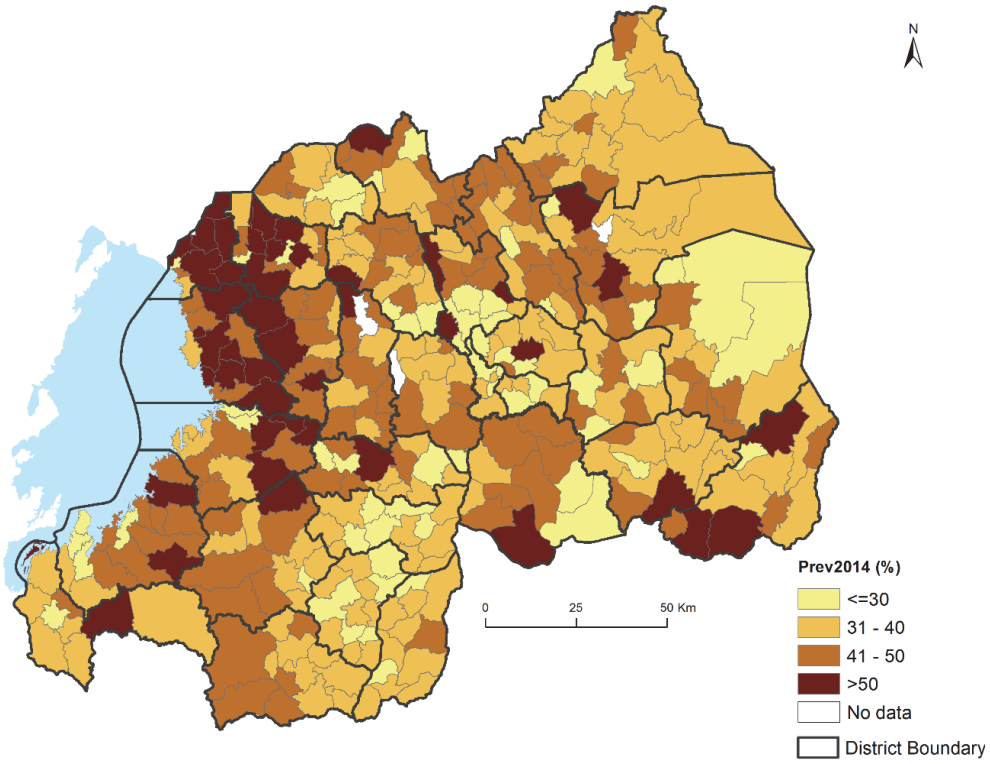


Figure 26. Stunting prevalence from CMHS data mapped on a health facility service area level

The routine collection of stunting prevalence data through HMIS data would offer the same high spatial resolution of stunting incidence.

Thus, if the stunting data from HMIS was correctly recorded, we believe having this incidence data would have the following advantages:

a. It would provide a regular picture of the stunting problem, and thus allow the mapping of stunting nationally on a high spatial resolution.
b. The high resolution mapping would help in targeting efforts to the most affected areas, maximize impact of the interventions, and provide a high temporal resolution of stunting for the monitoring of programs and interventions. Also, the HMIS stunting data would provide valuable complementary stunting data for the usual prevalence surveys such as the Rwanda DHS.

The collection of stunting incidence data on a health facility level in Rwanda is possible because:

c. The Rwanda health system is well organised in such a way that every mother or caregiver who would like to bring their children to the health centre can do so; caregivers can reach a health facility within a walking distance of 5 Km at most.
d. Community-health workers are close to the local population, and they actively encourage mothers to get their children routinely checked; and malnourished children are usually followed-up at the health centres.
e. As part of tackling the national stunting problem, in 2017, the Rwanda government introduced a new program whereby families who are in the lowest wealth category receive for free a micronutrient enriched complementary flour to feed to their children who are less than two years as a preventive measure of stunting (AIF, 2018). Thus, this program encourages mothers to bring their children to the health centres, where the enriched flours are distributed and the children's anthropometric measurements are also taken. The more stunted children are correctly followed-up in the system, the more the data will be rich and serve both the government of Rwanda and the scientific community.

6.4 Temporal variability of stunting in Rwanda

Since it was first included as an indicator in national surveys back in 1992, stunting in Rwanda has been on the decline at the national level. In the Demographic and Health Survey of 1992, the prevalence of stunting was 48% (ONP & Macro, 1994). However, after nearly 25 years, only a 10% reduction in stunting levels has been achieved, with 38% of children less than five years being stunted in 2015 (NISR et al., 2015). In addition, there is a temporal variability in stunting prevalence observed throughout the surveys. Since the first DHS survey in 1992, four other surveys have followed in 2000, in 2005, in 2010 and in 2015. Figure 27

shows the stunting prevalence in Rwanda since 1992 to 2015 per districts from 2000 to 2015 in comparison to the old province boundary used in 1992 to report stunting results. Year 2012 represents the data from the CFSVA survey.

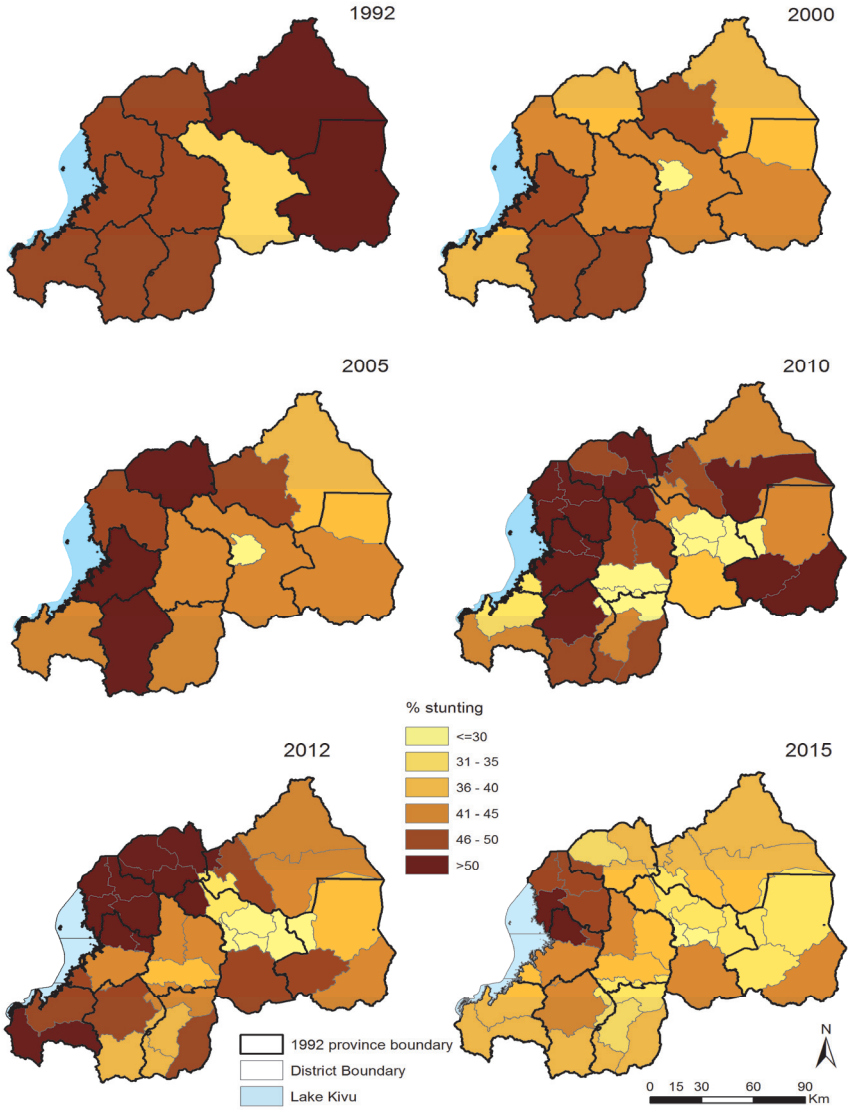


Figure 27. Stunting prevalence per districts in Rwanda from 1992 to 2015. Note: In 2000, the districts of Rwanda increased from 10 to 12 in 1992; and from 2010 and onwards, the districts were 30. All maps are based on DHS data, except for year 2012 which is based on CFSVA survey data.

Over the years especially in 2010, stunting became a priority for the government of Rwanda. The different policies and programs that were implemented managed to reduce stunting in some areas from more than 50% to less than 45%, which explains the contrast in the prevalence in 2010 and 2015. However, the current national prevalence of 38% is still very high. Thus, the spatial and temporal variability observed in stunting, although it is well explained by the known causes of stunting, we believe the mycotoxins contamination of flours, and the consequent exposure to children could be significantly playing a role. This is because, from all the causes of stunting studied in Rwanda so far, mycotoxins contamination of flours is probably the most volatile and unstable factor to measure and predict both in space and time. This is because of the extrinsic and intrinsic factors that play a role in the proliferation of fungi and the production of mycotoxins. Temperature, humidity, water activity, time of harvest, storage conditions all affect the production of aflatoxins in flours and the contamination can vary by sample, location, and particularly also by season.

Previous policies and programs in Rwanda have focused more on other immediate causes or risks of stunting such the age range of children, exclusive breastfeeding, hygiene and sanitation, diet diversification, micronutrient supplementation, access to health care and so on. However, the effect of mycotoxins exposure in children on stunting prevalence is still largely unknown. In our research, in addition to the known factors causing stunting, we demonstrated that the aflatoxins exposure was associated with low height-for-age in children in the Northern region of Rwanda. And on a national level due to the lack of data, we demonstrated that likely purchasing flours from markets located at the lower end of the food supply chain might increase the risk of exposure to aflatoxins. In addition, by modelling stunting, we believe the model spatial residuals observed explain and at the same time point to the unknown factors that cause stunting in Rwanda.

6.5 Implications for public health policies

Stunting is multifactorial, and addressing it requires a holistic approach considering all risk factors. To properly set policies however, some gaps need to be filled. First, to sustainably reduce stunting, dietary intake data in the country is needed. Thus, establishing a food composition table for Rwanda to monitor the intake of children, pregnant and lactating mothers should be among the priorities for the food and agriculture sector in Rwanda.. The nutrient intake of women of child bearing age is also of interest as they are an important population group in the prevention of stunting in future generations. Second, because of the risk of micronutrient malnutrition such as low dietary zinc intake for household relying on a plant-based diet, diet diversification requires to be more advocated for. Consumption of little dried fish available in Rwanda can be a good alternative to increase zinc content of the diets of children, especially in the Northern region of Rwanda. Third, the HMIS system currently operational in Rwanda offers great potential for efficient monitoring of stunting countrywide at detailed spatial scale. Thus, adjusting how the current stunting incidence cases are recorded at health facilities level throughout Rwanda by properly recording stunting cases, could provide the country with highly detailed spatial stunting data to target and follow-up on programs implementation. Also, there is also an urgent need to reduce the exposure to aflatoxins from complementary flours used to prepare porridges and stews for children. Thus, policies to prevent and monitor mycotoxins contamination along the food supply chain, and minimize the exposure to aflatoxins are needed. Lastly, because of the spatial heterogeneity of stunting in Rwanda, interventions to reduce stunting should be geographically targeted on a detailed spatial scale. This will be cost-effective and will provide maximum impact on the vulnerable populations.

6.6 Recommendation for future research

After studying the factors that determine the spatial pattern and variability of stunting in Rwanda by examining the dietary factors, mycotoxins exposure, environmental factors and the spatially structured and unexplained risk of stunting, several topics have evolved that could be considered for future research. Thus, the following recommendations are suggested:

1. To have a comprehensive understanding of the nutrient intake of children in Rwanda, a national survey on nutrient intake among children with a careful sampling design that considers the geographical differences in food intake is recommended. Also, a repeated 24-hr recall to estimate the usual intake of children should be considered. The results of the survey could provide a comprehensive overview of the spatial variability of the diets of children in Rwanda, and inform policy on gaps in nutrient intake that should be covered.

   On the other hand, as this research would require to use a food composition table, we suggest establishing a national food composition table prior to conducting a national nutrient intake survey to be a priority.

2. All the peanut flours samples analysed in this study were unfit for human consumption due to the high levels of aflatoxins found in them. Thus, there is a need to conduct a national mycotoxins survey in Rwanda that focuses not only on maize flour, which is still a significant source of exposure, but also on peanut flour. This national survey can be done in two ways. First the quantification of mycotoxins contamination can be done locally by studying the contamination levels in locally produced and consumed food products. Second, an examination of mycotoxins contamination along the food supply chain from farmer's field, to markets, and

from markets to the end-consumer. As maize and peanut flours are also imported from neighbouring countries, an examination of the extent of contamination from the customs reception to the end-consumer should be considered. In designing such a survey, there would be two important points to consider: the seasonality and the spatial-temporal dimension of mycotoxins contamination in foods. The seasonality would be very imperative to take into account as the climate and the environmental conditions are an important determinant of fungi growth and mycotoxins production. Also, the survey should be designed to be representative for the whole country, by conducting a spatial sample design. Mycotoxins levels in food stuff are highly dynamic, thus the space and time components needs to be examined in Rwanda.

3. Following the evidence that children are exposed to high levels of aflatoxins through the consumption of contaminated complementary flours, we recommend further studies on determining the exposure to aflatoxins using biomarkers in members of households especially children, pregnant and lactating mothers. This study is needed to accurately provide the extent of exposure to mycotoxins and affirm its causal link to stunting in children in Rwanda.

4. Research applying spatial analysis techniques to understand the spatio-temporal variation of stunting are needed. Also, studying stunting hotspots by conducting spatial cluster analyses on a health centre level could provide more insights into the specific locations in Rwanda that have persistent stunting hotspots. The correct stunting data collected on a health facility level through HMIS would easily facilitate these studies, and allow for an annual monitoring of not only stunting but also its risk factors. In addition, given the unexplained odds of stunting found in this research, further investigations applying geostatistical methods should be conducted

to understand the underlying determinants of the unexplained stunting burden that is persistent especially in the Western region of Rwanda.

Bibliography

Abdulkadar, A. H. W., Al-Ali, A. A., Al-Kildi, A. M., & Al-Jedah, J. H. (2004). Mycotoxins in food products available in Qatar. *Food Control, 15*(7), 543-548. doi:DOI 10.1016/j.foodcont.2003.08.008

ACC/SCN, & IFPRI. (2000). 4th Report-The World Nutrition Situation: Nutrition throughout the Life Cycle (pp. 140). Geneva: United Nations.

Adegoke, G. O., & Letum, P. (2013). Strategies for the Prevention and Reduction of Mycotoxins in Developing Countries. *INTECH*. doi:10.5772/52542

AIF. (2018). Solutions addressing malnutrition. Retrieved from https://africaimprovedfoods.com/products/institutional-products/

Allen, L., Benoist, B. d., & Hurrell, R. (2006). Guidelines on Food Fortification with Micronutrients. Geneva, Switzerland: World Health Organization and Food and Agriculture Organization of the United Nations.

Alvarado, B. E., Zunzunegui, M. V., Delisle, H., & Osorno, J. (2005). Growth trajectories are influenced by breast-feeding and infant health in an afro-colombian community. *J Nutr, 135*(9), 2171-2178. doi:10.1093/jn/135.9.2171

Amira M. Khan, Carducci, B., & Bhutta, Z. A. (2017). Low Birth Weight and Small for Gestational Age in the Context of 1,000 Days. In C. Karakochuk, K. Whitfield, Green, T., , & K. Kraemer (Eds.), *The Biology of the First 1,000 Days*. Boca Raton, FL: CRC Press.

Amuzie, C., Bandyopadhyay, R., Bhat, R. V., Black, R., Burger, H., Cardwell, K. F., . . . Wu, F. (2016). Mycotoxin control in low- and middle-income countries (pp. 54). Lyon, France.

Assare, R. K., Lai, Y. S., Yapi, A., Tian-Bi, Y. N., Ouattara, M., Yao, P. K., . . . N'Goran, E. K. (2015). The spatial distribution of Schistosoma mansoni infection in four regions of western Cote d'Ivoire. *Geospat Health, 10*(1), 345. doi:10.4081/gh.2015.345

AUC, & NEPAD. (2013). The Cost of Hunger in Rwanda, Social and Economic Impacts of Child Undernutrition in Rwanda, Implications on National Development and Vision 2020: African Union Commission (ACU), NEPAD Planning and Coordinating

Agency, Economic Commission for Africa (ECA), World Food Programme (WFP).

Balk, D., Storeygard, A., Levy, M., Gaskell, J., Sharma, M., & Flor, R. (2005). Child hunger in the developing world: An analysis of environmental and social correlates. *Food Policy, 30*(5-6), 584-611. doi:10.1016/j.foodpol.2005.10.007

Bergström, L. (1994). Nutrient Losses and Gains in the Preparation of Foods. Sweden: National Food Administration.

Berti, P. R., Kung'u, J. K., Tugirimana, P. L., Siekmans, K., Moursi, M., & Lubowa, A. (2011). Food and Nutrition Survey, Rwanda 2010-2011: Final Technical Report from Healthbridge to HarvestPlus. Kigali, Rwanda: HarvestPlus.

Bhat, R. V., & Vasanthi, S. (2003). Mycotoxin Food Safety Risk in Developing Countries. In L. J.Unnevehr (Ed.), *Food Safety in Food Security and Food Trade* (Vol. Brief 3, pp. 7-8). Washington, D.C.: International Food Research Institute.

Bhutta, Z. A., Ahmed, T., Black, R. E., Cousens, S., Dewey, K., Giugliani, E., . . . Child Undernutr Study, G. (2008). Maternal and Child Undernutrition 3 - What works? Interventions for maternal and child undernutrition and survival. *Lancet, 371*(9610), 417-440. doi:10.1016/s0140-6736(07)61693-6

Binagwaho, A., Condo, J., Wagner, C., Ngabo, F., Karema, C., Kanters, S., . . . Bizimana Jde, D. (2014). Impact of implementing performance-based financing on childhood malnutrition in Rwanda. *BMC Public Health, 14*, 1132. doi:10.1186/1471-2458-14-1132

Binagwaho, A., Farmer, P. E., Nsanzimana, S., Karema, C., Gasana, M., de Dieu Ngirabega, J., . . . Drobac, P. C. (2014). Rwanda 20 years on: investing in life. *The Lancet, 384*(9940), 371-375. doi:10.1016/S0140-6736(14)60574-2

Bizimana, J. P., Kienberger, S., Hagenlocher, M., & Twarabamenye, E. (2016). Modelling homogeneous regions of social vulnerability to malaria in Rwanda. *Geospat Health, 11*(1 Suppl), 404. doi:10.4081/gh.2016.404

Black, R. E., Allen, L. H., Bhutta, Z. A., Caulfield, L. E., de Onis, M., Ezzati, M., . . . Child Undernutrition Study, G. (2008). Maternal and child undernutrition: global and regional exposures and

health consequences. *Lancet, 371*(9608), 243-260. doi:10.1016/S0140-6736(07)61690-0

Black, R. E., Victora, C. G., Walker, S. P., Bhutta, Z. A., Christian, P., de Onis, M., . . . Child Nutrition Study, G. (2013). Maternal and child undernutrition and overweight in low-income and middle-income countries. *Lancet, 382*(9890), 427-451. doi:10.1016/S0140-6736(13)60937-X

Bognár, A. (2002). Tables on weight yield of food and retention factors of food constituents for the calculation of nutrient composition of cooked foods (dishes). Karlsruhe: Bundesforschungsanstalt für Ernährung (BFE).

Boniface, S., Atehnkeng, J., Mahuku, G., Bandyopadhyay, R., Nzamwita, M., & Henson, S. (2016). Mycotoxin contamination in Rwanda: quantifying the problem in maize and cassava in households and markets, biological control of aflatoxins and sensitization of targeted stakeholders based on a cost-benefit analysis. Kigali, Rwanda: Rwanda Agricultural Board.

Bosco, C., Alegana, V., Bird, T., Pezzulo, C., Bengtsson, L., Sorichetta, A., . . . Tatem, A. J. (2017). Exploring the high-resolution mapping of gender-disaggregated development indicators. *Journal of The Royal Society Interface, 14*(129). doi:10.1098/rsif.2016.0825

Branca, F., & Ferrari, M. (2002). Impact of micronutrient deficiencies on growth: the stunting syndrome. *Ann Nutr Metab, 46 Suppl 1*, 8-17. doi:10.1159/000066397

Brown, K. H. (2012). Assessing population zinc status with serum zinc concentration *IZiNCG Technical Brief No. 2*: International Zinc Nutrition Consultative Group (IZiNCG).

Brown, K. H., Peerson, J. M., Baker, S. K., & Hess, S. Y. (2009). Preventive zinc supplementation among infants, preschoolers, and older prepubertal children. *Food and Nutrition Bulletin, 30*(1), S12-S40. doi:10.1177/15648265090301s103

Brown, K. H., Wuehler, S. E., & Peerson, J. M. (2001). The Importance of Zinc in Human Nutrition and Estimation of the Global Prevalence of Zinc Deficiency. *Food and Nutrition Bulletin, 22*(2), 113-125. doi:10.1177/156482650102200201

Bunyavanich, S., Landrigan, C. P., McMichael, A. J., & Epstein, P. R. (2003). The impact of climate change on child health. *Ambul*

Pediatr, *3*(1), 44-52. doi:10.1367/1539-4409(2003)003<0044:TIOCCO>2.0.CO;2

Burgert, C. R., Colston, J., Roy, T., & Zachary, B. (2013a). Geographic displacement procedure and georeferenced data release policy for the Demographic and Health Surveys. DHS Spatial Analysis Reports No. 7. Calverton, Maryland, USA: ICF International.

Burgert, C. R., Colston, J., Roy, T., & Zachary, B. (2013b). Guidelines on the Use of DHS GPS Data. Spatial Analysis Reports No. 8. Calverton, Maryland, USA: ICF International.

CDC. (2004). Outbreak of aflatoxin poisoning-Eastern and Central Provinces, Kenya, January-July 2004. *MMWR Morb Mortal Wkly Rep*, *53*(34), 790-793.

Checkley, W., Buckley, G., Gilman, R. H., Assis, A. M., Guerrant, R. L., Morris, S. S., . . . Infection, N. (2008). Multi-country analysis of the effects of diarrhoea on childhood stunting. *Int J Epidemiol*, *37*(4), 816-830. doi:10.1093/ije/dyn099

Checkley, W., Buckley, G., Gilman, R. H., Assis, A. M., Guerrant, R. L., Morris, S. S., . . . Network, I. (2008). Multi-country analysis of the effects of diarrhoea on childhood stunting. *International Journal of Epidemiology*, *37*(4), 816-830. doi:10.1093/ije/dyn099

Chopra, M. (2006). Risk factors for undernutrition of young children in a rural area of South Africa. *Public Health Nutr*, *6*(07), 645-652. doi:10.1079/phn2003477

Condo, J. U., Gage, A., Mock, N., Rice, J., & Greiner, T. (2015). Sex differences in nutritional status of HIV-exposed children in Rwanda: a longitudinal study. *Trop Med Int Health*, *20*(1), 17-23. doi:10.1111/tmi.12406

Cope, M., & Elwood, S. (2009). *Qualitative GIS: A Mixed Methods Approach*: SAGE Publications Ltd.

Cotty, P. J., & Jaime-Garcia, R. (2007). Influences of climate on aflatoxin producing fungi and aflatoxin contamination. *Int J Food Microbiol*, *119*(1-2), 109-115. doi:10.1016/j.ijfoodmicro.2007.07.060

Dang, S., Yan, H., Yamamoto, S., Wang, X., & Zeng, L. (2004). Poor nutritional status of younger Tibetan children living at high altitudes. *Eur J Clin Nutr*, *58*(6), 938-946. doi:10.1038/sj.ejcn.1601915

de Onis, M., Blossner, M., & Borghi, E. (2012). Prevalence and trends of stunting among pre-school children, 1990-2020. *Public Health Nutr, 15*(1), 142-148. doi:10.1017/S1368980011001315

de Onis, M., Borghi, E., Arimond, M., Webb, P., Croft, T., Saha, K., . . . Flores-Ayala, R. (2019). Prevalence thresholds for wasting, overweight and stunting in children under 5 years. *Public Health Nutr, 22*(1), 175-179. doi:10.1017/S1368980018002434

de Onis, M., & Branca, F. (2016). Childhood stunting: a global perspective. *Matern Child Nutr, 12*(Suppl 1), 12-26. doi:10.1111/mcn.12231

de Onis, M., Martorell, R., Garza, C., & Lartey, A. (2006). WHO Child Growth Standards based on length/height, weight and age. *Acta Paediatrica, 95*, 76-85. doi:10.1080/08035320500495548

de Sherbinin, A. (2011). The biophysical and geographical correlates of child malnutrition in Africa. *Popul Space Place, 17*(1), 27-46. doi:10.1002/psp.599

Development Initiatives. (2018). 2018 Global Nutrition Report: Shining a light to spur action on nutrition. Bristol, UK: Development Initiatives.

Dewey, K. G., & Begum, K. (2011). Long-term consequences of stunting in early life. *Matern Child Nutr, 7 Suppl 3*, 5-18. doi:10.1111/j.1740-8709.2011.00349.x

Dewey, K. G., & Brown, K. H. (2003). Update on technical issues concerning complementary feeding of young children in developing countries and implications for intervention programs. *Food Nutr Bull, 24*(1), 5-28. doi:10.1177/156482650302400102

Dewey, K. G., & Huffman, S. L. (2009). Maternal, infant, and young child nutrition: combining efforts to maximize impacts on child growth and micronutrient status. *Food Nutr Bull, 30*(2 Suppl), S187-189. doi:10.1177/15648265090302S201

DHS, M. (2012). *Rwanda Demographic and Health Survey 2010*. Retrieved from

Dowell, S. F., Blazes, D., & Desmond-Hellmann, S. (2016). Four steps to precision public health. *Nature, 540*(7632), 189-191. doi:DOI 10.1038/540189a

Dzantiev, B. B., Byzova, N. A., Urusov, A. E., & Zherdev, A. V. (2014). Immunochromatographic methods in food analysis. *Trac-Trends in Analytical Chemistry, 55*, 81-93. doi:10.1016/j.trac.2013.11.007

Egal, S., Hounsa, A., Gong, Y. Y., Turner, P. C., Wild, C. P., Hall, A. J., . . . Cardwell, K. F. (2005). Dietary exposure to aflatoxin from maize and groundnut in young children from Benin and Togo, West Africa. *Int J Food Microbiol, 104*(2), 215-224. doi:10.1016/j.ijfoodmicro.2005.03.004

Engle-Stone, R., & Hess, S. Y. (2007). Quantifying the risk of zinc deficiency: Recommended indicators *Technical Brief No. 01*. University of California: IZiNCG.

ESRI. (2012). What is GIS? : Environmental Systems Research Institute.

FAO. (1991). Food, Nutrition and Agriculture Review-1-Food for the Future (pp. 72). Rome, Italy: Food and Agriculture Organization of the United Nations.

FAO. (2001). Manual on the application of the HACCP system in mycotoxin prevention and control (pp. 124). Rome, Italy: Food and Agriculture Organization of the United Nations.

FAO. (2004). Worldwide regulations for mycotoxins in food and feed in 2003 *FAO Food and Nutrition Paper 81*. Rome: Food and Agriculture Organization of the United Nations.

FAO. (2012). FAO/INFOODS Density Database Version 2.0. Rome, Italy: Food and Agriculture Organization of the United Nations.

FAO, IFAD, UNICEF, WHFP, & WHO. (2017). The State of Food Security and Nutrition in the World 2017. Building resilience for peace and food security. Rome, Italy: FAO.

FAO/INFOODS. (2012). Guidelines for Food Matching Version 1.2. Rome, Italy: Food and Agriculture Organization of the United Nations.

Ferguson, E. L., Gibson, R. S., Opare-Obisaw, C., Ounpuu, S., Thompson, L. U., & Lehrfeld, J. (1993). The zinc nutriture of preschool children living in two African countries. *J Nutr, 123*(9), 1487-1496. doi:10.1093/jn/123.9.1487

Gbashi, S., Madala, N. E., Adekoya, I., Adebo, O., De Saeger, S., De Boevre, M., & Njobeh, P. B. (2018). The socio-economic impact of mycotoxin contamination in Africa. In P. B. Njobeh (Ed.), *Fungi and Mycotoxins - Their Occurrence, Impact on Health and the Economy*

as well as Pre- and Postharvest Management Strategies [Working Title]: IntechOpen.

Gelfand. (1996). Model determination using sampling-based methods. In W. R. Gilks, S. Richardson, & D. Spiegelhalter (Eds.), *Markov Chain Monte Carlo in Practice*. USA.

Gething, P., Tatem, A., Bird, T., & Burgert-Brucker, C. R. (2015). Creating Spatial Interpolation Surfaces with DHS Data *Spatial Analysis Reports No. 11*. Rockeville, Maryland, USA: ICF International.

Gewa, C. A., Murphy, S. P., & Neumann, C. G. (2007). Out-of-home food intake is often omitted from mothers' recalls of school children's intake in rural Kenya. *Journal of Nutrition, 137*(9), 2154-2159. doi:10.1093/jn/137.9.2154

Gibson, R. S. (1994). Zinc nutrition in developing countries. *Nutr Res Rev, 7*(1), 151-173. doi:10.1079/NRR19940010

Gibson, R. S. (2006). Zinc: the missing link in combating micronutrient malnutrition in developing countries. *Proc Nutr Soc, 65*(1), 51-60. doi:10.1079/Pns2005474

Gibson, R. S. (2007). Determining the risk of zinc deficiency: Assessment of dietary zinc intake *IZiNCG Technical Brief No 3*. Davis, California: International Zinc Nutrition Consultative Group (IZiNCG).

Gibson, R. S., & Ferguson, E. L. (2008). An interactive 24-hour recall for assessing the adequacy of iron and zinc intakes in developing countries *HarvestPlus Technical Monograph* (pp. 160). Washington, D.C. and Cali, Colombia: International Food Policy Research Institute (IFPRI) and International Center for Tropical Agriculture (CIAT).

Gizachew, D., Szonyi, B., Tegegne, A., Hanson, J., & Grace, D. (2016). Aflatoxin contamination of milk and dairy feeds in the Greater Addis Ababa milk shed, Ethiopia. *Food Control, 59*(Supplement C), 773-779. doi:10.1016/j.foodcont.2015.06.060

Gnonlonfin, G. J., Hell, K., Adjovi, Y., Fandohan, P., Koudande, D. O., Mensah, G. A., . . . Brimer, L. (2013). A review on aflatoxin contamination and its implications in the developing world: a sub-Saharan African perspective. *Crit Rev Food Sci Nutr, 53*(4), 349-365. doi:10.1080/10408398.2010.535718

Gong, Y., Hounsa, A., Egal, S., Turner, P. C., Sutcliffe, A. E., Hall, A. J., . . . Wild, C. P. (2004). Postweaning exposure to aflatoxin results in impaired child growth: a longitudinal study in Benin, West Africa. *Environ Health Perspect, 112*(13), 1334-1338.

Gong, Y. Y., Egal, S., Hounsa, A., Turner, P. C., Hall, A. J., Cardwell, K. F., & Wild, C. P. (2003). Determinants of aflatoxin exposure in young children from Benin and Togo, West Africa: the critical role of weaning. *Int J Epidemiol, 32*(4), 556-562. doi:10.1093/ije/dyg109

GoR. (2013). Economic Development and Poverty Reduction Strategy 2013-2018 (pp. 77-83). Kigali: Government of Rwanda.

Grace, K., Davenport, F., Funk, C., & Lerner, A. M. (2012). Child malnutrition and climate in Sub-Saharan Africa: An analysis of recent trends in Kenya. *Applied Geography, 35*(1-2), 405-413. doi:DOI 10.1016/j.apgeog.2012.06.017

Grantham-McGregor, S., Cheung, Y. B., Cueto, S., Glewwe, P., Richter, L., Strupp, B., & International Child Development Steering, G. (2007). Developmental potential in the first 5 years for children in developing countries. *Lancet, 369*(9555), 60-70. doi:10.1016/S0140-6736(07)60032-4

Greenfield, H., & Southgate, D. A. T. (2003). *Food composition data: Production, Management and Use* (B. A. Burlingame & U. R. Charrondiere Eds.). Rome: Food and Agriculture Organization of the United Nations.

Groopman, J. D., Kensler, T. W., & Wild, C. P. (2008). Protective interventions to prevent aflatoxin-induced carcinogenesis in developing countries. *Annu Rev Public Health, 29*, 187-203. doi:10.1146/annurev.publhealth.29.020907.090859

Guerrant, R. L., DeBoer, M. D., Moore, S. R., Scharf, R. J., & Lima, A. A. M. (2012). The impoverished gut—a triple burden of diarrhoea, stunting and chronic disease. *Nature Reviews Gastroenterology &Amp; Hepatology, 10*, 220. doi:10.1038/nrgastro.2012.239

Guerrant, R. L., Oriá, R. B., Moore, S. R., Oriá, M. O., & Lima, A. A. (2008). Malnutrition as an enteric infectious disease with long-term effects on child development. *Nutrition Reviews, 66*(9), 487-505. doi:10.1111/j.1753-4887.2008.00082.x

Hagos, S., Hailemariam, D., WoldeHanna, T., & Lindtjørn, B. (2017). Spatial heterogeneity and risk factors for stunting among children

under age five in Ethiopia: A Bayesian geo-statistical model. *PLoS ONE, 12*(2), e0170785. doi:10.1371/journal.pone.0170785

Heimer, J., Staudacher, O., Steiner, F., Kayonga, Y., Havugimana, J. M., Musemakweri, A., . . . Mockenhaupt, F. P. (2015). Age-dependent decline and association with stunting of Giardia duodenalis infection among schoolchildren in rural Huye district, Rwanda. *Acta Tropica, 145*, 17-22. doi:10.1016/j.actatropica.2015.01.011

Hell, K., & Mutegi, C. (2011). Aflatoxin control and prevention strategies in key crops of Sub-Saharan Africa. *African Journal of Microbiology Research, 5*(5), 459-466.

Hotz, C., & Brown, K. H. (2004). Assessment of the risk of zinc deficiency in populations and options for its control. *Food and Nutrition Bulletin, 25*(1).

Hove, M., De Boevre, M., Lachat, C., Jacxsens, L., Nyanga, L. K., & De Saeger, S. (2016). Occurrence and risk assessment of mycotoxins in subsistence farmed maize from Zimbabwe. *Food Control, 69*, 36-44. doi:10.1016/j.foodcont.2016.04.038

Huey, S. L., & Mehta, S. (2016). Stunting: The Need for Application of Advances in Technology to Understand a Complex Health Problem. *EBioMedicine, 6*, 26-27. doi:10.1016/j.ebiom.2016.03.013

INSR, & Macro, O. (2006). Rwanda Demographic and Health Survey 2005. Calverton, Maryland, USA: Institut National de la Statistique du Rwanda (INSR), ORC Macro.

IOM. (2002/2005). Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amina Acids Washington DC: Institute of Medicine of the National Academy of Sciences.

IOM. (2006). Dietary Refence Intakes: The Essential Guide to Nutrient Requirements. Washington DC: Institute of Medicine of the National Academy of Sciences.

IZiNCG. (2004). International Zinc Nutrition Consultative Group (IZiNCG) technical document #1. Assessment of the risk of zinc deficiency in populations and options for its control. *Food Nutr Bull, 25*(1), S99-S203.

Jankowska, M. M., Lopez-Carr, D., Funk, C., Husak, G. J., & Chafe, Z. A. (2012). Climate change and human health: Spatial modeling of

water availability, malnutrition, and livelihoods in Mali, Africa. *Applied Geography, 33*(1), 4-15. doi:10.1016/j.apgeog.2011.08.009

Jolly, C. M., Bayard, B., Awuah, R. T., Fialor, S. C., & Williams, J. T. (2009). Examining the structure of awareness and perceptions of groundnut aflatoxin among Ghanaian health and agricultural professionals and its influence on their actions. *The Journal of Socio-Economics, 38*(2), 280-287. doi:10.1016/j.socec.2008.05.013

Kamala, A., Kimanya, M., Lachat, C., Jacxsens, L., Haesaert, G., Kolsteren, P., . . . De Meulenaer, B. (2017). Risk of Exposure to Multiple Mycotoxins from Maize-Based Complementary Foods in Tanzania. *J Agric Food Chem, 65*(33), 7106-7114. doi:10.1021/acs.jafc.6b03429

Kamika, I., Ngbolua, K. T. N., & Tekere, M. (2016). Occurrence of aflatoxin contamination in maize throughout the supply chain in the Democratic Republic of Congo. *Food Control, 69*(Supplement C), 292-296. doi:10.1016/j.foodcont.2016.05.014

Kandala, N.-B., Madungu, T. P., Emina, J. B. O., Nzita, K. P. D., & Cappuccio, F. P. (2011). Malnutrition among children under the age of five in the Democratic Republic of Congo (DRC): does geographic location matter? *BMC Public Health, 11*, 261-261. doi:10.1186/1471-2458-11-261

Kateera, F., Ingabire, C. M., Hakizimana, E., Kalinda, P., Mens, P. F., Grobusch, M. P., . . . van Vugt, M. (2015). Malaria, anaemia and under-nutrition: three frequently co-existing conditions among preschool children in rural Rwanda. *Malar J, 14*, 440. doi:10.1186/s12936-015-0973-z

Kaur, K., Gupta, R., Saraf, S. A., & Saraf, S. K. (2014). Zinc: The Metal of Life. *Comprehensive reviews in Food Science and Food Safety, 13*(4), 358-376. doi:Doi 10.1111/1541-4337.12067

Kazembe, L. N., Kleinschmidt, I., & Sharp, B. L. (2006). Patterns of malaria-related hospital admissions and mortality among Malawian children: an example of spatial modelling of hospital register data. *Malar J, 5*, 93. doi:10.1186/1475-2875-5-93

Khlangwiset, P., Shephard, G. S., & Wu, F. (2011). Aflatoxins and growth impairment: a review. *Crit Rev Toxicol, 41*(9), 740-755. doi:10.3109/10408444.2011.575766

Kimanya, M. E. (2015). The health impacts of mycotoxins in the eastern Africa region. *Current Opinion in Food Science, 6*, 7-11. doi:10.1016/j.cofs.2015.11.005

King, J. C. (2011). Zinc: an essential but elusive nutrient. *American Journal of Clinical Nutrition, 94*(2), 679S-684S. doi:10.3945/ajcn.110.005744

Kinyoki, D. K., Berkley, J. A., Moloney, G. M., Kandala, N.-B., & Noor, A. M. (2015). Predictors of the risk of malnutrition among children under the age of 5 years in Somalia. *Public Health Nutrition, 18*(17), 3125-3133. doi:10.1017/S1368980015001913

Krasevec, J., An, X., Kumapley, R., Bégin, F., & Frongillo, E. A. (2017). Diet quality and risk of stunting among infants and young children in low- and middle-income countries. *Maternal & Child Nutrition, 13*(S2), e12430. doi:10.1111/mcn.12430

Kristina Michaux, Aranka Anema, Timothy Green, Lauren Smith, Judy McLean, Abiud Omwega, . . . Ahimbisibwei, M. (2014). Home fortification with micronutrient powders: Lessons learned from formative research across six countries. *Sight and Life, 28*, 26-35.

Lander, R., Enkhjargal, T. S., Batjargal, J., Bolormaa, N., Enkhmyagmar, D., Tserendolgor, U., . . . Gibson, R. S. (2010). Poor dietary quality of complementary foods is associated with multiple micronutrient deficiencies during early childhood in Mongolia. *Public Health Nutrition, 13*(9), 1304-1313. doi:10.1017/S1368980009991856

Lattanzio, V. M. T., Guarducci, N., Powers, S., Ciasca, B., Pascale, M., & von Holst, C. (2018). Validation of a lateral flow immunoassay for the rapid determination of aflatoxins in maize by solvent free extraction. *Analytical Methods, 10*(1), 123-130. doi:10.1039/c7ay02249b

Legg, C. (2008). *A Spatial Analysis of Child Nutrition in West Africa. ReSAKSS Working Paper no. 23*. Retrieved from Ibadan, Nigeria:

Li, W., Powers, S., & Dai, S. Y. (2014). Using commercial immunoassay kits for mycotoxins: 'joys and sorrows'? *World Mycotoxin Journal, 7*(4), 417-430. doi:10.3920/wmj2014.1715

Liu, Y., & Wu, F. (2010). Global burden of aflatoxin-induced hepatocellular carcinoma: a risk assessment. *Environ Health Perspect, 118*(6), 818-824. doi:10.1289/ehp.0901388

Logrieco, A., Miller, J., Eskola, M., Krska, R., Ayalew, A., Bandyopadhyay, R., . . . Leslie, J. (2018). The Mycotox Charter:

Increasing Awareness of, and Concerted Action for, Minimizing Mycotoxin Exposure Worldwide. *Toxins, 10*(4), 149.

Lowe, N. M., Fekete, K., & Decsi, T. (2009). Methods of assessment of zinc status in humans: a systematic review. *Am J Clin Nutr, 89*(6), 2040S-2051S. doi:10.3945/ajcn.2009.27230G

Lu, C., Mejia-Guevara, I., Hill, K., Farmer, P., Subramanian, S. V., & Binagwaho, A. (2016). Community-Based Health Financing and Child Stunting in Rural Rwanda. *Am J Public Health, 106*(1), 49-55. doi:10.2105/AJPH.2015.302913

Lukmanji, Z., Hertzmark, E., Mlingi, N., Assey, V., Ndossi, G., & Fawzi, W. (2008). Tanzania Food Composition Tables (Vol. 1st Edition). Dar es Salaam, Tanzania: Muhimbili University of Health and Allied Sciences (MUHAS), Tanzania Food and Nutrition Centre (TFNC), Harvard School of Pubic Health (HSPH).

Lunn, D. J., Thomas, A., Best, N., & Spiegelhalter, D. (2000). WinBUGS - A Bayesian modelling framework: Concepts, structure, and extensibility. *Statistics and Computing, 10*(4), 325-337. doi:10.1023/a:1008929526011

Lyambabaje, A., Ndahindwa, V., Bigirimana, Z., Iyakaremye, D., Umugwaneza, M., Matsiko, E., & Ahishakiye, J. (2015). Rwanda National Nutrition Screening: Key findings (year 2014). Kigali, Rwanda: College of Medicine and Health Sciences (CMHS), University of Rwanda.

Maheswaran, R., & Craglia, M. (2004). *GIS in Public Health Practice*: CRC Press LLC.

Martorell, R. (1999). The nature of child malnutrition and its long-term implications. *Food and Nutrition Bulletin, 20*(3).

Martorell, R., Khan, L. K., & Schroeder, D. G. (1994). Reversibility of stunting: epidemiological findings in children from developing countries. *Eur J Clin Nutr, 48 Suppl 1*, S45-57.

Marx, S., Phalkey, R., Aranda-Jan, C. B., Profe, J., Sauerborn, R., & Hofle, B. (2014). Geographic information analysis and web-based geoportals to explore malnutrition in Sub-Saharan Africa: a systematic review of approaches. *BMC Public Health, 14*, 1189. doi:10.1186/1471-2458-14-1189

Mason, A., Richardson, S., Plewis, I., & Best, N. (2012). Strategy for modelling non-random missing data mechanisms in observational

studies using Bayesian methods. *Journal of Official Statistics, 28*(2), 279-302.

Mayo-Wilson, E., Imdad, A., Junior, J., Dean, S., & Bhutta, Z. A. (2014). Preventive zinc supplementation for children, and the effect of additional iron: a systematic review and met-analysis. *British Medical Journal Open, 4*.

McDonald, C. M., & Thorne-Lyman, A. L. (2017). The Importance of the First 1,000 Days-An Epidemiological Perspective. In C. Karakochuk, K. Whitfield, Green, T., , & K. Kraemer (Eds.), *The Biology of the First 1,000 Days*. Boca Raton, FL: CRC Press.

McNeil, A. J., & Wendin, J. P. (2007). Bayesian inference for generalized linear mixed models of portfolio credit risk. *Journal of Empirical Finance, 14*(2), 131-149. doi:10.1016/j.jempfin.2006.05.002

Mendelsohn, J., Jarvis, A., Robertson, T., & Nyiratuza, M. (2016). Rwanda: The Measure of a Land. Kigali: Vital Signs.

MIGEPROF. (2018). Rwanda Country Strategic Review of Food and Nutrition Security. Kigali: Ministry of Gender and Family Promotion.

MINAGRI, NISR, & WFP. (2016). Rwanda 2015-Comprehensive food security and vulnerability analysis. Kigali, Rwanda.

MINALOC, MOH, & MINAGRI. (2013). Rwanda National Food and Nutrition Policy. Kigali: Government of Rwanda.

MINALOC, MOH, & MINAGRI. (2014a). National Food and Nutrition Policy 2013-2018.

MINALOC, MOH, & MINAGRI. (2014b). National Food and Nutrition Strategic Plan 2013-2018.

MOH. (2005). *National Nutrition Policy*. Kigali: Ministry of Health.

MOH. (2013). *"A Thousand Days in the Land of a Thousand Hills" Nutrition Communication Initiative, Strategic Plan*. Kigali, Rwanda: Ministry of Health.

Mukabutera, A., Thomson, D. R., Hedt-Gauthier, B. L., Basinga, P., Nyirazinyoye, L., & Murray, M. (2016). Risk factors associated with underweight status in children under five: an analysis of the 2010 Rwanda Demographic Health Survey (RDHS). *BMC Nutrition, 2*(1), 40. doi:10.1186/s40795-016-0078-2

Mupfasoni, D., Karibushi, B., Koukounari, A., Ruberanziza, E., Kaberuka, T., Kramer, M. H., . . . Fenwick, A. (2009). Polyparasite

helminth infections and their association to anaemia and undernutrition in Northern Rwanda. *PLoS Negl Trop Dis, 3*(9), e517. doi:10.1371/journal.pntd.0000517

Mupunga, I., Mngqawa, P., & Katerere, D. R. (2017). Peanuts, Aflatoxins and Undernutrition in Children in Sub-Saharan Africa. *Nutrients, 9*(12). doi:10.3390/nu9121287

Murphy, S. P., Gewa, C., Grillenberger, M., & Neumann, C. G. (2004). Adapting an international food composition table for use in rural Kenya. *Journal of Food Composition and Analysis, 17*(3–4), 523-530. doi:10.1016/j.jfca.2004.03.019

Mutegi, C., Wagacha, M., Kimani, J., Otieno, G., Wanyama, R., Hell, K., & Christie, M. E. (2013). Incidence of aflatoxin in peanuts (Arachis hypogaea Linnaeus) from markets in Western, Nyanza and Nairobi Provinces of Kenya and related market traits. *J Stored Prod Res, 52*(Supplement C), 118-127. doi:10.1016/j.jspr.2012.10.002

Mwalwayo, D. S., & Thole, B. (2016). Prevalence of aflatoxin and fumonisins (B1 + B2) in maize consumed in rural Malawi. *Toxicol Rep, 3*, 173-179. doi:10.1016/j.toxrep.2016.01.010

Navarro, H., Navarro, S., & Finkelman, S. (2012). Hermetic and modified atmosphere storage of shelled peanuts to prevent free fatty acid and aflatoxin formation. *IOBC/WPRS Bulletin, 81*, 183-192.

Ngirabega, J. D. D., Hakizimana, C., Wendy, L., Donnen, P., & Dramaix-Wilmet, M. (2010). Amélioration de la gestion d'un programme de suivi de la croissance à base communautaire des enfants en milieu rural au Rwanda. *Revue d'Épidémiologie et de Santé Publique, 58*(2), 111-119. doi:10.1016/j.respe.2009.11.003

Nikoi, E., & Anthamatten, P. (2013). An examination of environmental correlates with childhood height-for-age in Ghana. *Public Health Nutr, 16*(1), 46-53. doi:10.1017/S1368980012001346

Nishimwe, K., Wanjuki, I., Karangwa, C., Darnell, R., & Harvey, J. (2017). An initial characterization of aflatoxin B1 contamination of maize sold in the principal retail markets of Kigali, Rwanda. *Food Control, 73*(Part B), 574-580. doi:10.1016/j.foodcont.2016.09.006

NISR, & MINECOFIN. (2012). Fourth Rwanda Population and Housing Census (2012 RPHC) Census Atlas: National Institute of Statistics, Ministry of Finance and Economic Planning

NISR, MOH, & ICF. (2010). Rwanda Demographic and Health Survey 2010. Calverton, Maryland, USA: National Institute of Statistics of Rwanda (NISR), Ministry of Health (MOH), ICF International.

NISR, MOH, & ICF. (2015). Rwanda Demographic and Health Survey 2014-15. Rockville, Maryland, USA: National Institute of Statistics of Rwanda (NISR), Ministry of Health (MOH), ICF International.

Nriagu, J. (2018). Zinc Deficiency in Human Health. *Encyclopedia of Environmental Health*, 789-800. doi:10.1016/b978-0-12-409548-9.11433-2

Nyandwi, E., Veldkamp, A., Amer, S., Karema, C., & Umulisa, I. (2017). Schistosomiasis mansoni incidence data in Rwanda can improve prevalence assessments, by providing high-resolution hotspot and risk factors identification. *BMC Public Health, 17*(1), 845. doi:10.1186/s12889-017-4816-4

Nyandwi, E., Veldkamp, T., & Amer, S. (2016). Regional climate sensitivity of wetland environments in Rwanda: the need for a location-specific approach. *Regional Environmental Change, 16*(6), 1635-1647. doi:10.1007/s10113-015-0905-z

Nyandwi, E., Veldkamp, T., Osei, F. B., & Amer, S. (2017). Spatio-temporal dynamics of schistosomiasis in Rwanda between 2001 and 2012: impact of the national Neglected Tropical Disease control programme. *Geospat Health, 12*(1), 514. doi:10.4081/gh.2017.514

Nyinawabali, F., Njobeh, P. B., & Dutton, M. F. (2013). *A survey of fungi and mycotoxins in selected food commodities from Rwanda (Thesis).* University of Johannesburg, Johannesburg, RSA.

Okoth, S. A., & Ohingo, M. (2004). Dietary aflatoxin exposure and impaired growth in young children from Kisumu District, Kenya: Cross sectional study. *Afr J Health Sci, 11*(1-2), 43-54.

Onis, M. (2007). WHO Child Growth Standards based on length/height, weight and age. *Acta Paediatr, 95*, 76-85. doi:10.1111/j.1651-2227.2006.tb02378.x

ONP, & Macro. (1994). Enquête Démographique et de Santé Rwanda 1992. In B. Barrère, J. Schoemaker, M. Barrère, T. Habiyakare, A. Kabagwira, & M. Ngendakumana (Eds.). Kigali, Rwanda: Office National de la Population, Marco International Inc.

Onyango, A. W., Esrey, S. A., & Kramer, M. S. (1999). Continued breastfeeding and child growth in the second year of life: a prospective cohort study in western Kenya. *Lancet, 354*(9195), 2041-2045. doi:10.1016/S0140-6736(99)02168-6

Ortiz, J. (2014). *Infant feeding, nutritional status and mycotoxin exposure of children in the Ecuadorian highlands. Ph.D. dissertation.* Ghent University, Ghent.

Osgood-Zimmerman, A., Millear, A. I., Stubbs, R. W., Shields, C., Pickering, B. V., Earl, L., . . . Hay, S. I. (2018a). Mapping child growth failure in Africa between 2000 and 2015. *Nature, 555*(7694), 41-+. doi:10.1038/nature25760

Osgood-Zimmerman, A., Millear, A. I., Stubbs, R. W., Shields, C., Pickering, B. V., Earl, L., . . . Hay, S. I. (2018b). Mapping child growth failure in Africa between 2000 and 2015. *Nature, 555*, 41. doi:10.1038/nature25760

https://www.nature.com/articles/nature25760#supplementary-information

Owaga, E., Muga, R., Mumbo, H., & Aila, F. (2011). Chronic dietary aflatoxins exposure in Kenya and emerging public health concerns of impaired growth and immune suppression in children. *Intl J of Bio Chem Sci, 5*(3), 1325-1336. doi:10.4314/ijbcs.v5i3.72287

Pitt, J. I. (2000). Toxigenic fungi and mycotoxins. *Br Med Bull, 56*(1), 184-192.

Rabassa, M., Skoufias, E., & Jacoby, H. (2014). Weather and Child Health in Rural Nigeria. *Journal of African Economies*. doi:10.1093/jae/eju005

Rand, W. M., Pennington, J. A. T., Murphy, S. P., & Klensin, J. C. (1991). *Compiling Data for Food Composition Data Bases*. Hong Kong: United Nation University Press.

Ricketts, T. C. (2003). Geographic Information Systems and Public Health. *Annual Review of Public Health, 24*, 1-6.

Roche, M. L., Gyorkos, T. W., Blouin, B., Marquis, G. S., Sarsoza, J., & Kuhnlein, H. V. (2016). Infant and young child feeding practices and stunting in two highland provinces in Ecuador. *Matern Child Nutr*. doi:10.1111/mcn.12324

RSB. (2012). Cooked Packaged Maize Specification. Kigali, Rwanda: Rwanda Standards Board.

Rutstein, S. O., & Rojas, G. (2006). Guide to DHS Statistics *Demographic and Health Surveys Methodology*. Calverton, Maryland, USA: Measure DHS/ICF International.

Sakisaka, K., Wakai, S., Kuroiwa, C., Flores, L. C., Kai, I., Mercedes Arago´n, M., & Hanada, K. (2006). Nutritional status and associated factors in children aged 0–23 months in Granada, Nicaragua. *Public Health, 120*(5), 400-411. doi:10.1016/j.puhe.2005.10.018

Salam, R. A., MacPhail, C., Das, J. K., & Bhutta, Z. A. (2013). Effectiveness of Micronutrient Powders (MNP) in women and children. *BMC Public Health, 13*(3), S22. doi:10.1186/1471-2458-13-s3-s22

Salgueiro, M. J., Zubillaga, M. B., Lysionek, A. E., Caro, R. A., Weill, R., & Boccio, J. R. (2002). The role of zinc in the growth and development of children. *Nutrition, 18*(6), 510-519. doi:10.1016/S0899-9007(01)00812-7

Sanchis, V., & Magan, N. (2004). 8 - Environmental conditions affecting mycotoxins. In N. Magan & M. Olsen (Eds.), *Mycotoxins in Food* (pp. 174-189): Woodhead Publishing.

Semba, R. D., de Pee, S., Sun, K., Sari, M., Akhter, N., & Bloem, M. W. (2008). Effect of parental formal education on risk of child stunting in Indonesia and Bangladesh: a cross-sectional study. *The Lancet, 371*(9609), 322-328. doi:10.1016/S0140-6736(08)60169-5

Shephard, G. S. (2008a). Determination of mycotoxins in human foods. *Chemical Society Reviews, 37*(11), 2468-2477. doi:10.1039/b713084h

Shephard, G. S. (2008b). Impact of mycotoxins on human health in developing countries. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess, 25*(2), 146-151. doi:10.1080/02652030701567442

Skoufias, E., & Vinha, K. (2012). Climate variability and child height in rural Mexico. *Econ Hum Biol, 10*(1), 54-73. doi:10.1016/j.ehb.2011.06.001

Smith, L. E., Stoltzfus, R. J., & Prendergast, A. (2012). Food chain mycotoxin exposure, gut health, and impaired growth: a conceptual framework. *Advances in nutrition (Bethesda, Md.), 3*(4), 526-531. doi:10.3945/an.112.002188

Spatial Data Repository, & DHS Program. (2015). *Modeled Surfaces. RW2015DHS_CNNUTSCHA2_MS_v01*. Retrieved from spatialdata.dhsprogram.com.

Sperandei, S. (2014). Understanding logistic regression analysis. *Biochem Med (Zagreb), 24*(1), 12-18. doi:10.11613/BM.2014.003

Stadlmayr, B., Charrondiere, U. R., Enujiugha, V. N., Bayili, R. G., Fagbohoun, E. G., Samb, B., . . . Burlingame, B. (2012). West African Food Composition Table/Table de composition des aliments d'Afrique de l'Ouest. Rome: The Food and Agriculture Organization of the United Nations, International Networkd of Food Data System, West Africa Health Organisation, Biodiversity International.

Stewart, C. P., Iannotti, L., Dewey, K. G., Michaelsen, K. F., & Onyango, A. W. (2013). Contextualising complementary feeding in a broader framework for stunting prevention. *Matern Child Nutr, 9 Suppl 2*, 27-45. doi:10.1111/mcn.12088

Strosnider, H., Azziz-Baumgartner, E., Banziger, M., Bhat, R. V., Breiman, R., Brune, M. N., . . . Wilson, D. (2006). Workgroup report: public health strategies for reducing aflatoxin exposure in developing countries. *Environ Health Perspect, 114*(12), 1898-1903. doi:10.1289/ehp.9302

Udomkun, P., Mutegi, C., Wossen, T., Atehnkeng, J., Nabahungu, N. L., Njukwe, E., . . . Bandyopadhyay, R. (2018). Occurrence of aflatoxin in agricultural produce from local markets in Burundi and Eastern Democratic Republic of Congo. *Food Sci Nutr, 6*(8), 2227-2238. doi:10.1002/fsn3.787

Udomkun, P., Wiredu, A. N., Nagle, M., Bandyopadhyay, R., Muller, J., & Vanlauwe, B. (2017). Mycotoxins in Sub-Saharan Africa: Present situation, socio-economic impact, awareness, and outlook. *Food Control, 72*, 110-122. doi:10.1016/j.foodcont.2016.07.039

Umereweneza, D., Kamizikunze, T., & Muhizi, T. (2018). Assessment of mycotoxins types in some foodstuff consumed in Rwanda. *Food Control, 85*(Supplement C), 432-436. doi:10.1016/j.foodcont.2017.10.015

Umugwaneza, M., Wentzel-Viljoen, E., Vorster, H., & Havemann-Nel, L. (2017). *The development of food based dietary guidelines (FBDGs) for 6*

to 23 month old Rwandan children (PhD Thesis). North-West University, Potchefstroom, South Africa.

UN. (1993). Malnutrition and Infection-A review *Nutrition policy discussion paper No. 5*. Geneva, Switzerland: United Nations Administrative Committee on Coordination − Subcommittee on Nutrition (ACC/SCN).

UN. (2005). Designing Household Survey Samples: Practical Guidelines (U. N. S. Division, Trans.) *Studies in Methods, Series F No.98*. New York: United Nations.

UN. (2015). Transforming our World: The 2030 Agenda for Sustainable Development. New York, USA: United Nations.

UNICEF. (1990). Strategies for improved nutrition of children and women in developing countries *A UNICEF Policy Review*. New York: United Nations Children's Fund.

UNICEF. (2013). Improving child nutrition-the achievable imperative for global progress. New York: United Nations Children's Fund.

UNICEF, WHO, & Group, W. B. (2019). Levels and trends in child malnutrition: key findings of the 2019 Edition of the Joint Child Malnutrition Estimates-UNICEF Regions: United Nations Children's Fund (UNICEF), World Health Organization (WHO), International Bank for Reconstruction and Development/The World Bank.

USAID/Rwanda. (2006). Rwanda HMIS Assessment Report. In R. International (Ed.). NC, USA.

USDA. (2015). USDA National Nutrient Database for Standard Reference, Release 28 (Vol. September 2015). Beltsville, Maryland: US Department of Agriculture, Agricultural Research Service, Nutrient Data Laboratory.

USDA, & GIPSA. (2015). GIPSA Performance verified Mycotoxin Test Kits: United States Department of Agriculture.

USDHEW, & FAO. (1968). Food Composition Table for Use in Africa. Maryland: United States Department of Health, Education, and Welfare; Food and Agriculture Organization of the United Nations.

Uwiringiyimana, V., Amer, S., & Veldkamp, A. Stunting spatial pattern in Rwanda: an examination of the demographic, socio-economic and environmental determinants (submitted).

Uwiringiyimana, V., Ocke, M. C., Amer, S., & Veldkamp, A. Exposure to aflatoxins from maize and peanut flours and stunting in young children from the Northern region of Rwanda (submitted).

Uwiringiyimana, V., Ocke, M. C., Amer, S., & Veldkamp, A. (2018a). Data on child complementary feeding practices, nutrient intake and stunting in Musanze District, Rwanda. *Data Brief, 21*, 334-342. doi:10.1016/j.dib.2018.09.084

Uwiringiyimana, V., Ocke, M. C., Amer, S., & Veldkamp, A. (2018b). Predictors of stunting with particular focus on complementary feeding practices: A cross-sectional study in the northern province of Rwanda. *Nutrition, 60*, 11-18. doi:10.1016/j.nut.2018.07.016

Vasco, C., Ruales, J., & Kamal-Eldin, A. (2008). Total phenolic compounds and antioxidant capacities of major fruits from Ecuador. *Food Chemistry, 111*(4), 816-823. doi:10.1016/j.foodchem.2008.04.054

Vatcheva, K. P., Lee, M., McCormick, J. B., & Rahbar, M. H. (2016). Multicollinearity in Regression Analyses Conducted in Epidemiologic Studies. *Epidemiology (Sunnyvale, Calif.), 6*(2), 227. doi:10.4172/2161-1165.1000227

Victora, C. G., Adair, L., Fall, C., Hallal, P. C., Martorell, R., Richter, L., . . . Child Undernutrition Study, G. (2008). Maternal and child undernutrition: consequences for adult health and human capital. *Lancet, 371*(9609), 340-357. doi:10.1016/S0140-6736(07)61692-4

Victora, C. G., de Onis, M., Hallal, P. C., Blossner, M., & Shrimpton, R. (2010). Worldwide timing of growth faltering: revisiting implications for interventions. *Pediatrics, 125*(3), e473-480. doi:10.1542/peds.2009-1519

Vilcins, D., Sly, P. D., & Jagals, P. (2018). Environmental Risk Factors Associated with Child Stunting: A Systematic Review of the Literature. *Annals of Global Health, 84*(4), 551. doi:10.29024/aogh.2361

Villers, P. (2014). Aflatoxins and safe storage. *Frontiers in Microbiology, 5*, 158. doi:10.3389/fmicb.2014.00158

Voss, P. R., Long, D. D., Hammer, R. B., & Friedman, S. (2006). County child poverty rates in the US: a spatial regression approach. *Popul Res Policy Rev, 25*(4), 369-391. doi:10.1007/s11113-006-9007-4

Wagacha, J. M., & Muthomi, J. W. (2008). Mycotoxin problem in Africa: current status, implications to food safety and health and possible management strategies. *Int J Food Microbiol, 124*(1), 1-12. doi:10.1016/j.ijfoodmicro.2008.01.008

Wamani, H., Åstrøm, A. N., Peterson, S., Tumwine, J. K., & Tylleskär, T. (2007). Predictors of poor anthropometric status among children under 2 years of age in rural Uganda. *Public Health Nutr, 9*(3), 320-326. doi:10.1079/PHN2005854

Weise, A. S. (2012). WHA Global Nutrition Targets 2025: Stunting Policy Brief: World Health Organization.

Weiss, R. (1996). An Approach to Bayesian Sensitivity Analysis. *Journal of the Royal Statistical Society, 58*(4), 739-750.

Wessells, K. R., & Brown, K. H. (2012). Estimating the global prevalence of zinc deficiency: results based on zinc availability in national food supplies and the prevalence of stunting. *PLoS ONE, 7*(11), e50568. doi:10.1371/journal.pone.0050568

WFP, & CDC. (2005). A Manual: Measuring and Interprating Malnutrition and Mortality. Rome, Italy: World Food Program (WFP) and Center for Disease Control and Prevention (CDC).

WFP, MINAGRI, & NISR. (2012). Comprehensive Food Security and Vulnerability Analysis: World Food Programme.

WHO. (1995). Physical status: The Use and Interpretation of Anthropometry. Geneva, Switzerland: World Health Organization.

WHO. (2001). Global strategy for infant and young child feeding: The optimal duration of exclusive breastfeeding. Geneva, Switzerland: World Health Organization.

WHO. (2003). Mycotoxins Fact Sheet No5. Congo, Brazzavile: WHO Regional Office for Africa.

WHO. (2006a) Mycotoxins in African Foods: Implications to Food Safety and Health. *Vol. 2*: AFRO Food Safety Newsletter.

WHO. (2006b). WHO child growth standards: methods and development. Geneva.

WHO. (2010). Indicators for assessing infant and young child feeding practices: Part 2 Measurement. Geneva, Switzerland: The World Health Organization.

WHO. (2011). WHO Anthro for personal computers, version 3.2.2: Software for assessing growth and development of the world's children. Geneva: World Health Organization.

WHO. (2012). Nutrition of women in in the preconception period, during pregnancy and the breastfeeding period. Geneva, Switzerland: World Health Organization.

WHO/FAO. (2004). Vitamin and mineral requirements in human nutrition 2nd edition (2nd ed., Vol. 2nd edition). Bangkok, Thailand: World Health Organization, Food and Agriculture Organization of the United Nations.

Wild, C. P., & Gong, Y. Y. (2010). Mycotoxins and human disease: a largely ignored global health issue. *Carcinogenesis, 31*(1), 71-82. doi:10.1093/carcin/bgp264

Worboys, M. F. (2003). *GIS: A Computing Perspective*: Taylor & Francis e-Library.

Yang, Y. Y., Kaddu, G., Ngendahimana, D., Barkoukis, H., Freedman, D., Lubaale, Y. A. M., . . . Bakaki, P. M. (2018). Trends and determinants of stunting among under-5s: evidence from the 1995, 2001, 2006 and 2011 Uganda Demographic and Health Surveys. *Public Health Nutrition, 21*(16), 2915-2928. doi:10.1017/S1368980018001982

Yue, A., Marsh, L., Zhou, H., Medina, A., Luo, R., Shi, Y., . . . Rozelle, S. (2016). Nutritional Deficiencies, the Absence of Information and Caregiver Shortcomings: A Qualitative Analysis of Infant Feeding Practices in Rural China. *PLoS ONE, 11*(4), e0153385. doi:10.1371/journal.pone.0153385

Appendix 2. Questionnaire for dietary intake and mycotoxins survey

Dietary intake and mycotoxins exposure survey

Title of project

Stunting spatial pattern in Rwanda

Researcher
Vestine Uwiringiyimana

Participant name:_____

Participant ID: PI_I_I_I

Interviewer Name: _____

Interviewer ID: I_I_I

Date of Interview: I_I_I-I_I_I-20 I_I_I

Date of Intake: I_I_I-I_I_I-20 I_I_I

Household code: HI_I_I_I

Time started

I_I_I: I_I_I (am/pm)

GPS Location

EL: I__I__I__I__I m

LA: I__I__I__I__I__I__I__I

LO: I__I__I__I__I__I__I__I

Cell: _____

Village: _____

District: _____

Sector:_____

Information sheet and consent form

[Please fill in below]

Has the respondent signed the informed consent?	YES □	NO □ (If NO interview ends here)
Is there a child under two years in the household?	YES □	NO □ (If NO interview ends here)

GENERAL INFORMATION

1	**Child name & ID**	Name: …… ID: |__|__|__|	
2	**Sex of child**	Male □	Female □
3	**Date of birth of child (day/month/year)**	……/……../……. (day/month/year)	
	Weight of child (kg)	Weight 1: |__|__|.|__| (kg)	Weight 2: |__|__|.|__| (kg)
	Height of child (cm)	Height 1: |__|__|__|.|__| (cm)	Height 2: |__|__|__|.|__| (cm)
4	**Age of mother/caretaker**		
	Weight of mother (kg)	Weight 1: |__|__|__|. |__|	Weight 2: |__|__|__|. |__|
	Height of mother (cm)	Height: |__|__|__|.|__| (cm)	
5	**Marital status**	Married (monogamy)……..□ Married (polygamy)……..□ Single……□	Divorced……□ Widowed……□ Other (specify)……
6	**Relationship with child**	Mother……□ Stepmother……□ Grandmother……□	Auntie……□ Other

			(specify)…….
7	**Level of education**	Illiterate……□ Primary school……..……□ Post-primary (vocational)……□	Secondary school……..….□ Tertiary……..……□ Other (specify)……
8	**What is your religion?**	Catholic……..□ Protestant……□ Adventist……□ Muslim……□	Traditional……..……..□ No religion……..□ Other (specify)……
9	**What was the birth weight of your child?**	Birth weight: |__|__|.|__| (kg)	
10	**Was the child breastfed exclusively during the first 6 months?**	YES □	NO □
	10.1. If NO, what other foods/drinks did you give to your baby apart from breast milk?	Plain water ……□ Cow milk ……..□ Traditional herbal mixture……..……..□ Sugary water ……..□	Fruit juice ……..……..□ Porridge ……..□ Other, specify……
11	**How old was your baby when s/he drank or ate semi-solid food for the first time?**	|_____| (record the child age in months)	
12	**What was the reason that triggered you to offer your baby the drink/food mentioned in Q10.1?**	Inadequate breast milk ……..□ Work ……..□ Pre-lacteal feeding……□ Other, specify……	
13	**Is your child breastfeeding now? (tick)**	YES □	NO □

	13.1. If YES, how frequent did your child breastfeed yesterday?	Once a day……..□ Twice a day ……..□ Three times/day ……..□	More than 3 times/day ..□
	13.2. If NO, at what age (month) was your child completely weaned?	Age (months)……	
14	**When did you introduce complementary foods for the first time to your child?**	Age (months)……	
15	**Was your child given any vitamin A capsule in the last 6 months?**	YES □	NO □
16	**Has your child received deworming tablets in the last 6 months?**	YES □	NO □
17	**Have you used multiple micronutrient powders to add to the food of your child in the last 4 weeks?**	YES □	NO □
	17.1. If yes, how many times did you add MNP in the foods for your child?	Less than twice/ week …□ Twice /week……□	3times /week ……..□ More than 3 times /week…… …..□
18	**How many young children aged less than 5 years do you have?**	|_____| (number of children <5 years)	
19	**How many children <2 years live in the household?**	|_____| (number of children <2years)	
20	**How many lactating mothers live in the household?**	|_____| (number of lactating mothers)	
21	**How many pregnant mothers live in the household?**	|_____| (number of pregnant mothers)	
22	**How many household members do eat and sleep every day in your house?**	|_____| (number of household members)	
23	**With what activity does your household earn income? (tick where appropriate)**	None……□ Commerce……..□ Agriculture……..□	Domestic work……..□ Formal employment……□

			Other (specify)……..
24	**Does your household have access to *agricultural* land?**	YES □	NO □
	24.1. If yes, what is the ownership status?	Self-owned……..□ Hired……□	Public land……□ Other (specify)……..□
	24.2. What type of crops do you grow on your farm?	□Cereals (specify)…… □Roots & tubers…… □Legumes (specify)…… □Fruits (specify)…… □Vegetables (specify)…… □Other, specify……	
25	**Does your household own any livestock?**	YES □	NO □
	25.1. If YES, what is the number of livestock owned in the household?	Cow……..|____| Goat……|____| Sheep……|____| Pig……|____|	Rabbit……..|____| Chicken……|____| Other, specify……..
26	**What is the wealth category (*ubudehe*) of your household?**	Richest……□ Richer ……□ Rich ……□ Poor……..□	Poorer……..□ Poorest……..□ Don't know…… □

27	**What is the main source of drinking water for members of this household?**	Piped water Public tap……□ Piped into dwelling……□ Tube well or borehole……□ Dug well Protected well……..□ Unprotected well…..□	Water from spring Protected……..…..□ Unprotected……..…□ Rainwater……..……□ Surface water (river/ dam/ lake/stream/irrigation channel)……..……□ Other (specify)……..
28	**What do you do usually to make the water safe to drink?**	Boil……□ Add bleach/chlorine……..□ Strain through a cloth……□	Let it stand & settle…….□ Use water filter……□ Other (specify)……
29	**How much time does it take to go and collect water?**	Less than 30 min ……□ Between 30-60 min ….□ Between 1-2 hours……□	More than 2 hours ……□ Not applicable ……..□
30	**Does your household have a kitchen garden?**	YES □	NO □
	30.1. If YES, what types of crops are grown in the kitchen garden?	Amaranth……□ Spinach……..□ Cabbage……..□	Carrots……..……□ Celery……..……□ Other, specify……..
31	**Does your household grow biofortified crops?**	YES □	NO □
	31.1. If YES, which biofortified crops?	Beans……..□ Maize……..□ Banana plantain……..□	Orange sweet potatoes…..□ Other, specify……

32	**Does your household use improved seeds?**	YES □	NO □
	32.1. Which improved seeds do you use specifically?	Maize ……..□ Wheat……□ Barley……□	Plantain ……..□ Other, specify……..
33	**Does your household use inorganic fertilizers?**	YES □	NO □
34	**In the past 4 weeks, which illness did you child suffer from?**	Fever ……..□ Diarrhoea ……..□ Vomiting ……..□	Malaria……□ Cough ……..□ Other, specify …….
	34.1. How frequent does your child suffer from the illness (es) mentioned in Q34?	Less than once a month……□ Once a month ……..□	Twice a month ……..□ More than 2times/month ……..□ Other, specify…….
	34.2. In case more than one illness in Q34, use this space to record the information	Illness	Frequency

24-HR RECALL DIETARY INTAKE QUESTIONNAIRE

DAY RECALLED (tick where appropriate)						
MON	**TUE**	**WED**	**THUR**	**FRI**	**SAT**	**SUN**

Instructions

Reminder to the mother/caregiver: The interview is to enable us find out what your child has eaten the previous day. All that your child has eaten including drinks, foods, sauces, spices and snacks will need to be recalled. There is no right or wrong answer in this interview; you only need to tell me what your child has actually eaten. All your responses will be treated with outmost confidentiality. **When I ask you about your child, always refer to the one aged between 6-24 months.**

Interview steps

1. Quick List of Food Items

[Quickly record all food and drink items consumed by the child in the previous day in the "Quick List of Food Items"]

Please tell me everything your child ate or drank all day yesterday, from 6 o'clock yesterday morning until 6 o'clock this morning. Include everything that the child ate and drank at home and away, even snacks [Do not interrupt unnecessarily]

[When respondent stops, ask:] ***Anything else?***

Now, I'm going to ask you more details about the foods and drinks you just listed. I want you to tell me "when", "which occasion", "what", "how much" and "where" your child ate all his/her foods yesterday.
When I ask about amounts, please use any of cups, mugs, bowls or plate to estimate the amount of food/drink your child ate or drank at yesterday. I will use our graduated food models to estimate the final amount eaten by the child and the weight equivalent of the food/drink.
When you remember anything else s/he ate as we go along, please tell me.

2. Column 1
    a. Time: ***About what time did your child eat/drink the food?***
    b. Occasion: ***What would you call this occasion?*** (Refer to indications below the form; i.e. breakfast, mid-morning, etc.)
    c. Place eaten: ***Where did your child eat the food?*** (Probe: home, neighbour's home, etc.)

3. Column 2
    a. Food or drink: Transfer the Quick list of food items to the column of Food or drink. Cross out the food in Quick list
    b. Description of food/drink and cooking method & ingredients: Ask about the details of cooking methods and ingredients

What was the food the child ate/drank made of?
What food ingredients were in the meal or dish?
Did it have any other ingredients? [If yes] ***what were the ingredients?***
[Request a detailed description of each food/drink item on the form, refer to the table below. If applicable request food labels when the mother/caregiver cannot answer the ingredients and record information on the label in the appropriate columns]

Food type	**Required detailed information**
Meat	Kind of meat (beef, goat, lamb, rabbit), organ meat (liver, kidney, heart), description of cut, raw or cooked weight, method of cooking, lean or lean plus fat, bone in or not (waste factor)
Fish and seafood	Kind of fish or seafood, raw or cooked weight, method of cooking, amount of bones, skin or shell (waste factor)
Poultry	Kind of poultry, parts of pieces eaten (e.g. breast, thigh), raw or cooked weight, method of cooking, meat plus skin or meat only, bones (waste factor)
Fats	Kind of fat, brand name (if possible), vegetable fat, ghee (raw unprocessed/ processed), brand name (if commercial product) i.e. margarine
Milk products	Kind of dairy product, brand name (if commercial product), percentage of fat (as butter fat or milk fat), liquid vs. powdered milk, cheese (kind of cheese i.e. whole milk, percentage fat if known, brand name if commercial product)
Bread	Type of grain (whole wheat, white bread), homemade or bought, size: standard or unusual, toasted or not, topping and condiments, brand name (if commercial product)

Cereal, pasta or rice	Type of grain, whole or refined (e.g., sorghum, millet, etc.), milled or polished (for rice), brand name, raw or cooked weight, enriched or not, cereal plus milk, method of cooking (e.g., porridge alone, with added milk/sugar)
Vegetables	Fresh (green leafy vegetables- spinach, amaranth, cassava leaves, etc.), other vegetables (leek, celery, onions, paprika), frozen or canned (e.g., canned tomatoes); peeled or unpeeled; method of cooking; topping (butter, palm oil, ghee, vegetable oil)
Fruits	Fresh, stewed, frozen or canned; peeled or unpeeled; type of liquid (heavy, light): sweetened or unsweetened; waste factor (e.g. peel, stone)
Pulses	Beans, bean types, peas, lentils, fresh or dry, French beans, method of cooking
Beverages, soup	Fresh or frozen, canned or bottled, fruit juice: sweetened or unsweetened; added vitamins or minerals (e.g. vitamin C); tea: green tea, black tea, with added milk/sugar, brand name (if commercial product); soups: homemade or canned, dilutant (milk or water), proportion of dilutant: concentrate (e.g., 1:1), recipe; brand name (if commercial)
Street foods from vendors	Food (e.g., doughnuts, samosa, fried/roasted peanuts), brand name (if commercial product), condiments added (eggs, food colour), method of cooking, vendor's name/location
Mixed dishes	Product name, homemade or commercial, recipe ingredients, cooking method
Herbs, spices	Name; fresh or dried; salt, pepper, maggi cubes, royco cubes; other spices, fish powder, pilipili, onions

[For homemade mixed dishes, record on the 24hr recall form, the following additional details:

- Name of mixed dish (local and general);
- Descriptive list of all ingredients in descending order of quantity;
- Amount of each raw ingredient (excluding water);
- Method of preparation and cooking;
- Total amount of cooked dish (in grams or mLs); and
- Amount of the mixed dish consumed by the respondent in the same units (record under "amount eaten by child")]

4. Column 3: Source of foods

Ask about the food source

Where did you obtain the food? Or where did the food come from? (Refer to indications below the form; 1.1. from family farm, purchase, restaurant, etc.)

5. Column 4: Amount eaten by child

Ask about the amount of the food/drink eaten/drank by the child

How much did the child eat this food?

[Remind mother to use own cups/mugs/bowls or plate to estimate the amount eaten by the child]

6. Go to the next food item on the Quick List. [Skip this step and go to step 7 when all foods in the Quick List have been asked]

7. Food break and review: ***Now let's see what the child ate between occasions and if I have everything:***
   a. ***What was the first food or drink your child had after waking up yesterday? (Time?) (First occasion?)***
   b. ***Now at (Time) for (This occasion) your child had (Foods), did s/he have anything else?***
   c. ***Did your child have anything to eat or drink between (Time) (This occasion) and (Time) when s/he had (Next occasion)? Such as snacks, fruits or drinks?***

Repeat 7b and 7c for each occasion except last occasion. For last occasion, go to 7d

   d. ***Now at (Time) for (Last occasion) your child had (Foods), did s/he have anything else?***
   e. ***Did you child have anything to eat or drink after (Time) (Last occasion) but before 6am this morning?***
   f. ***Did s/he have anything to eat or drink between midnight last night and waking up today?***

I would like you to try to remember anything else your child ate or drank yesterday, that you haven't already told me about, including anything s/he ate or drank while his/her meal was being prepared or while waiting to eat.

Did your child eat these foods?

Forgotten foods list	
Snacks	Doughnuts (mandazi), samosa, peanuts (roasted), bread, biscuits (brand name if commercial))
Fruits	Banana, tree tomato, avocado, orange, papaya, passion fruit, etc.
Drinks	Fanta, water, fruit juice (brand name if commercial), fruit juice powder (i.e. supadip)
Sweets	Any type

[If yes, ask for the details (portion size, brand name, place/time eaten, source of food, etc.); if no, continue to question 8]

24-hr Dietary Intake Questionnaire

Quick list of food items	**Column 1**			**Column 2**		**Column 3**	**Column 4**	**Coder use only**	
	Time (--:--)	**Occasion[1]**	**Place eaten**	**Food or Drink**	**Description of food/drink, Preparation/cooking method and Ingredients**	**Source of food[2]**	**Amount eaten by child**	**Weight equivalent (g)**	**Food code**

Occasion[1]: 1. Breakfast 2. Mid-morning 3. Lunch 4. Afternoon 5. Dinner 6. Night/early dawn 7. Other (specify): ……

Source of food[2]: 1. Homemade (1.1. From own farm; 1.2. Purchase; 1.3. Gift) 2. Restaurant 3. Friend/relative home 4. Other (specify) ……..

24-hr Dietary Intake Questionnaire

Quick list of food items	Column 1			Column 2		Column 3	Column 4	Coder use only	
	Time (--:--)	Occasion[1]	Place eaten	Food or Drink	Description of food/drink, Preparation/cooking method and Ingredients	Source of food[2]	Amount eaten by child	Weight equivalent (g)	Food code

Occasion[1]: 1. Breakfast 2. Mid-morning 3. Lunch 4. Afternoon 5. Dinner 6. Night/early dawn 7. Other (specify): ……

Source of food[2]: 1. Homemade (1.1. From own farm; 1.2. Purchase; 1.3. Gift) 2. Restaurant 3. Friend/relative home 4. Other (specify) ……..

24-hr Dietary Intake Questionnaire

Quick list of food items	Column 1			Column 2		Column 3	Column 4	Coder use only	
	Time (--:--)	Occasion[1]	Place eaten	Food or Drink	Description of food/drink, Preparation/cooking method and Ingredients	Source of food[2]	Amount eaten by child	Weight equivalent (g)	Food code

Occasion[1]: 1. Breakfast 2. Mid-morning 3. Lunch 4. Afternoon 5. Dinner 6. Night/early dawn 7. Other (specify): ……

Source of food[2]: 1. Homemade (1.1. From own farm; 1.2. Purchase; 1.3. Gift) 2. Restaurant 3. Friend/relative home 4. Other (specify) ……..

8. Does yesterday represent a typical day in your household? ***(Tick where appropriate)***	YES □	NO □
Was it a feast day?	YES □	NO □
Was it a market day?	YES □	NO □
Was it a fasting day?	YES □	NO □
If other, please specify…….		
9. Are you the person most responsible for preparing meals for your child in the household?	YES □	NO □
Other, please specify……..		
10. Is the food the child ate yesterday similar to his/her usual food intake?	YES □	NO □
If NO, please give the reason……		
11. Who fed the child yesterday? Please specify relationship	……	
12. Yesterday was the food for the child cooked in a separate pot?	YES □	NO □
If YES, is this usual?	YES □	NO □
13. ***Was the food dished out for the child in his/her own plate?***	YES □	NO □
For either YES or NO, is this usual?	YES □	NO □
14. Was the child sick yesterday?	YES □	NO □
If YES, did the sickness affect appetite?	YES □	NO □
If YES, how?	Increase □ Decrease □	Not sure □
If YES, please specify the sickness ……..		

[*I am now going to ask you a few additional questions on maize and peanut flour*]

Mycotoxins exposure questionnaire

[*Answer the following questions as honestly as possible*]

1. Do you feed your child (tick where appropriate) a. Maize? b. Peanuts?	 Yes□ Yes □	 No □ No □
2. How many times per week do you feed your child? (tick where appropriate)	**Maize**	**Peanut**
Less than twice/ week	□	□
Twice /week	□	□
Three times /week	□	□
More than 3 times/week	□	□
3. In which form of maize and peanuts do you mostly use to prepare them for your child?	**Maize** Flour ……..□ Maize comb Roasted……□ Boiled……□ Other, specify……..	**Peanuts** Flour……..□ Roasted ……□ Other, specify……..
4. Does the maize or maize flour that you use to cook meals for your child the same that is used to cook meals in your household?	YES □	NO □
5. Basing on question 4, does this applies also to peanuts and peanuts flour?	YES □	NO □
6. What is the source of maize and peanut? (tick where appropriate)	**Maize**	**Peanuts**
a. Farm (own production)	□	□
b. Purchase from the market	□	□
c. Other, specify	……..	……..

7. Do you sort the maize before milling or other form of processing?	YES □	NO □
8. Do you sort the peanuts before milling or other form of processing?	YES □	NO □
9. Explain how you sort the maize/peanuts (state below)		
Sorting of maize:	**Sorting of peanuts:**	
10. How and where do you normally store the maize and peanuts grains and flour? (answer separately for maize and peanuts)		
Maize:	**Peanuts:**	
11. For how long do you store the maize and peanut flour? (answer separately for maize and peanuts)		
12. Do the maize and peanuts grains get spoiled sometimes while in storage?	YES □	NO □
13. Do peanuts grains get spoiled sometimes while in storage?	YES □	NO □
14. If YES, can you please tell me the signs of spoilage in grains and the flours? (answer separately for maize and peanuts)		
15. What do you do with spoiled maize grains/flour?		
16. What do you do with spoiled peanut grains/flour?		

Sample collection of maize flour and peanut flour

Now, kindly ask the mother/caregiver to give you a portion of the flour of maize and peanut that she uses to cook and feed her child; 200 to 500 g for maize and 200 to 500g for peanut. Remind her that this is to be taken for laboratory analysis of mycotoxins. Note: if the mother does not have milled flour but still has maize and peanuts grains, go ahead and collect them.

Fill in the table below:

Sample collected	**YES (tick below)**		**NO (tick below)**	**Quantity collected (grams)**
Maize	Flour □	Grains □	□	
Peanut	Flour □	Grains □	□	

Thank you for your cooperation!!

Time ended: |_|_|: |_|_| (am/pm)

24-hr Dietary Intake Questionnaire

Interviewer Observation Form

[Do not read these questions to the respondent]

A. Did you or the respondent have difficulty with this intake interview? (Circle where applies)
   a. YES
   b. NO

B. What was the reason of this difficulty?

C. Any additional remarks

FOR OFFICE USE ONLY	
Date received:	Data entry: YES NO
Completed questionnaire: YES NO	Entered by:
Missing data make up: YES NO	Re-entry: YES NO
Verified by:	Entered by:

Summary

Stunting or impaired linear growth is still a significant public health problem in most developing countries. Stunting occurs when a child is not growing in length or height in accordance with his or her potential. Globally, 22% of children less than five years are stunted. In Africa, the East-Africa and the Middle Africa regions have the highest stunting levels, of 36.7% and 32.5% respectively. Rwanda particularly faces unacceptable levels of stunting with 38% of children less than five years stunted. According to the thresholds of stunting in a population set by the World Health Organization, a prevalence of more than 30% is considered very high. Stunting is a significant problem affecting countries' economic and social productivity, mainly because of its effects during the first two years of life, are mostly irreversible during adulthood. Stunting affects severely cognitive development, health and adult economic development. Geographically, stunting prevalence varies spatially and temporally on a national and regional scale. The usual reported prevalence on a national or sub-national level overshadows the spatial heterogeneity in stunting that exists at finer geographical scale within countries. Understanding the drivers of the spatial heterogeneity in stunting is paramount to set targeted interventions and achieve sustainable results. In Rwanda, the spatial heterogeneity in stunting is observed throughout the whole country, with the Northern and Western provinces highly affected compared to other provinces. Although the Rwandan government has implemented different strategies to tackle the problem of chronic malnutrition, in some districts, the high level of stunting and its spatial variation remains unexplained. This dissertation aimed at understanding the drivers of the spatial pattern of stunting in Rwanda by focusing on complementary feeding practices, poor diet quality, mycotoxins exposure and environmental factors. A two-scale level approach was conducted; the first part focused on conducting a local case-study to understand the determinants of stunting by focusing on complementary feeding

practices and mycotoxins exposure in the district of Musanze. The second part involved up-scaling the analysis on a national level by applying GIS for spatially mapping and modelling stunting.

The first stage of the research studied the determinants of stunting in the Northern region of Rwanda with particular focus on the complementary feeding practices and dietary zinc intake. The findings showed that stunting was very high (44%) in the region. Most of the children were still breastfed, but their complementary diet was deficient in essential nutrients for growth and development due to a predominantly plant-based diet. The linear regression model showed that the main factors associated with height-for-age in children were age of children, exclusive breastfeeding, deworming tablets use in the previous six months, the caregiver BMI and dietary zinc intake. The logistic regression model showed that with every increase in age by one month, the risk of stunting increased by 20% in the study population. On the contrary, the odds of stunting reduced significantly when the child had received deworming tablets or had been exclusively breastfed. This study was among the few in Rwanda that assessed the dietary intake of children in Rwanda, especially the dietary zinc intake. A dietary diversification strategy of including locally available and affordable animal source foods in the diet of children is recommended to increase the nutrient quality of the children's diet.

The second stage of the study determined the aflatoxins level content of complementary flours (namely maize and peanut), estimated the exposure to aflatoxins in children and studied its association with stunting observed in the same study population. The levels of aflatoxins in maize and peanut flours used in the complementary feeding of children were very high, exceeding the regulatory limit for aflatoxins in East-Africa for peanut flour. Thus, maize and peanut flours fed to children as part of the weaning diet are a source of high exposure to

aflatoxins in children in Musanze District. The exposure to aflatoxins was significantly associated with stunting after adjusting for the identified predictors of stunting in stage one of the research. The result confirmed our hypothesis that the aflatoxins exposure in children in Rwanda is associated with stunting, and could thus, be one of the missing links to the continued observed high levels of stunting in some regions. More evidence is needed on the extent of exposure to aflatoxins in Rwandan children. Policies and programs to reduce and eventually minimize the daily exposure to aflatoxins in children are urgently needed.

The third stage carried out a spatially disaggregated analysis of stunting determinants by taking into account not only socio-economic factors but also biophysical factors and a proxy measure of mycotoxins exposure. Child and maternal factors that emerged as predictors of stunting were age, being male, birthweight, height of mothers and secondary education or higher. The examination of biophysical factors revealed that the elevation of a household cluster was negatively associated with height-for-age of children, implying the influence of remoteness on the health and nutrition of children. The elevation factor can influence accessibility to foods and health care services, which in turn are known to influence child growth. Because of the lack of spatially detailed data on mycotoxins in Rwanda, a proxy measure of mycotoxins exposure was used by considering households clusters served by rural markets located at the end of the food supply chain as having a high risk of exposure to mycotoxins. This proxy measure was found to be associated with height-for-age, for household clusters served by a rural market within a 10 Km radius. Our result call for more research on the extent of mycotoxins contamination along the food supply chain in Rwanda and the impact of the exposure to aflatoxins on stunting in children.

The fourth stage of this research focused on modelling the spatial pattern of stunting in Rwanda using a Bayesian geostatistical model. The risk factors of stunting were studied by considering stunting spatial dependency. Also, spatial residuals were predicted to identify the risk of stunting, not accounted for by child-related covariates previously included in the model. In the spatial model, child age, child sex, birthweight, exclusive breastfeeding and having had diarrhoea in two weeks that preceded the survey, mother BMI, mother education, wealth index, household flooring and access to improved water source were predictors of stunting. The prediction of spatial residual effects of stunting showed a spatially explicit pattern of unexplained high and low risk of stunting in Rwanda, especially the Western region. Interventions to reduce stunting should be geographically targeted by taking into account the spatial heterogeneity in stunting prevalence across the country. The pattern of the spatial residuals corresponded to the proxy factor of exposure to mycotoxins and elevation. Thus, further studies are needed to look into the unknown spatially explicit factors that are correlated with the sustained high risk of stunting.

Overall, this research has confirmed that the complementary diet of children in Musanze district is poor in essential nutrients, especially micronutrients such as zinc. Also, complementary feeding practices determine stunting in the same district. The research confirmed that aflatoxins exposure in children is associated with stunting. On a national level, below the usual reported district prevalence, stunting spatial pattern in Rwanda is still very heterogeneous. Mycotoxins exposure through the food supply chain was associated with stunting. Finally, considering the spatial dependency in stunting spatial pattern showed the risk factors of stunting and the unexplained spatially explicit pattern of the risk of stunting.

Samenvatting

Achterblijvende lengtegroei ('stunting') als gevolg van chronische ondervoeding bij kinderen is een belangrijk volksgezondheidsprobleem in de meeste ontwikkelingslanden. Wereldwijd treft dit 22% van alle kinderen jonger dan vijf jaar. In Afrika is de prevalentie van lineaire groeiretardatie het hoogst in Oost-Afrika (37%) en in Midden-Afrika (33%). Rwanda heeft een onaanvaardbaar hoge prevalentie van 38%. De Wereldgezondheidsorganisatie beschouwd een prevalentie van meer dan 30% als zeer hoog. Groeiretardatie uit zich niet alleen in een geringe lengte, maar leidt ook tot beperkte mentale en fysieke ontwikkeling, tot verminderde arbeidsproductiviteit en is daarmee een gezondheidsprobleem dat de economische en sociale productiviteit op nationaal niveau negatief beïnvloedt. Lineaire groeiachterstand en de gevolgen daarvan zijn irreversibel.

Achterblijvende lineaire groei wordt gewoonlijk gerapporteerd op nationaal of sub nationaal niveau. Het probleem is echter dat de prevalentie van groeiretardatie in een bepaald (studie) gebied gewoonlijk niet geografisch uniform is maar juist varieert in tijd en ruimte. In Rwanda is de prevalentie van achterblijvende lengtegroei eveneens ruimtelijk heterogeen waarbij met name de noordelijke en westelijke provincies gekenmerkt worden door een (zeer) hoge prevalentie. Hoewel de Rwandese regering verschillende strategieën heeft geïmplementeerd om chronische ondervoeding aan te pakken, blijven de hoge prevalentie en de significante ruimtelijke verschillen vooralsnog onverklaard. Het is daarom van groot belang om een beter inzicht te verkrijgen in de factoren die bijdragen aan deze ruimtelijke heterogeniteit om in de toekomst geografisch beter toegesneden beleidsinterventies te kunnen ontwikkelen.

Dit proefschrift richtte zich op het verkrijgen van een beter inzicht in de onderliggende factoren en ruimtelijke variabiliteit van achterblijvende

lengtegroei met specifieke aandacht voor voedingskwaliteit, aanvullende voeding (naast borstvoeding), blootstelling aan mycotoxinen, en sociaaleconomische- en geofysische omgevingsfactoren. Het onderzoek is uitgevoerd op twee schaalniveaus. Op lokaal niveau is een casestudie opgezet met als doel de voornaamste determinanten van achterblijvende lengtegroei te identificeren. De casus richtte zich op voedingskwaliteit, aanvullende voeding, en blootstelling aan mycotoxinen in het district Musanze, in de noordelijke provincie van Rwanda. Het tweede deel van het onderzoek bestond uit het opschalen van de analyse naar nationaal niveau waarbij de ruimtelijke variabiliteit van achterblijvende lengtegroei gedetailleerd in kaart werd gebracht om daarna te modelleren hoe achterblijvende lengtegroei is gerelateerd aan sociaaleconomische- en geofysische omgevingsfactoren.

Uitkomsten van de *eerste fase* van het onderzoek toonden aan dat de prevalentie van achterblijvende lengtegroei in de regio Musanze zeer hoog was (44%). De meeste kinderen kregen borstvoeding, maar hun aanvullende voeding bevatte (te) weinig micronutriënten, essentiële voedingsstoffen voor groei en ontwikkeling, als gevolg van een voornamelijk plantaardig dieet. Lineaire regressieanalyse toonde aan dat groeiretardatie voornamelijk was gerelateerd aan: leeftijd, exclusieve borstvoeding, gebruik van ontwormingstabletten in de voorgaande zes maanden, BMI van de verzorger, en de inname van zink via de voeding. Logistische regressie toonde aan dat bij elke verhoging van de leeftijd met een maand, het risico op groeiretardatie in de onderzoekspopulatie met 20% toenam. De kans op achterblijvende lineaire groei nam aanzienlijk af wanneer het kind was behandeld met ontwormingstabletten of uitsluitend borstvoeding had gekregen. De lokale casus in Musanze is een van de weinige studies die ook de inname van zink via de voeding bestudeerde. Op grond van de casus uitkomsten is het beleidsadvies een strategie te ontwikkelen gericht op diversificatie van de voeding, waarbij plaatselijk beschikbare en

betaalbare voedingsmiddelen van dierlijke oorsprong in de voeding van kinderen worden opgenomen.

In de *tweede fase* van het onderzoek is het aflatoxinegehalte bepaald in mais- en pindameel dat gebruikt werd als bijvoeding voor kinderen. De onderzoeksvraag hierbij was of blootstelling aan aflatoxinen gerelateerd is aan lineaire groeiachterstand. De gemeten aflatoxinegehalten in maïs- en met name pindameel bleken zeer hoog en overschreden (verre) de wettelijke grenswaarden voor aflatoxinen in Oost-Afrika. Statistische analyse toonde aan dat blootstelling aan aflatoxinen, in aanvulling op de in de eerste onderzoeksfase geïdentificeerde risicofactoren, inderdaad significant bijdraagt aan lineaire groeiachterstand. Aangezien deze resultaten gebaseerd zijn op een casus van relatief beperkte omvang wordt verder onderzoek naar de relatie tussen aflatoxine inname en groeiretardatie aanbevolen. Uitkomsten van dit onderzoek geven evenwel duidelijk aan dat beleid gericht op het voorkomen van blootstelling aan aflatoxinen via de voedselketen noodzakelijk is.

De *derde fase* van het onderzoek betreft een analyse, op nationaal niveau, van de ruimtelijke variabiliteit van achterblijvende lengtegroei en de relatie met sociaaleconomische kenmerken, biofysische omgevingsfactoren, en een proxy factor voor het schatten van mycotoxinen expositie via voedingsmiddelen. Het gebruik van een proxy factor was noodzakelijk vanwege het gebrek aan ruimtelijk gedetailleerde gegevens over het voorkomen van mycotoxinen in voedingsmiddelen in Rwanda. De proxy factor is gebaseerd op de mate waarin huishoudens toegang hebben tot stedelijke of rurale markten. De veronderstelling is dat huishoudens die worden bediend door meer afgelegen rurale markten aan het einde van de voedselvoorzieningsketen een hoger risico hebben op mycotoxinen expositie dan huishoudens die hun voedingsmiddelen kunnen

betrekken van meer centraal gelegen markten hoger in de hiërarchie van de voedselvoorzieningsketen.

Sociaaleconomische kenmerken positief geassocieerd met groeiretardatie waren: leeftijd, mannelijk geslacht, laag geboortegewicht, beperkte lengte van de moeder en een laag percentage van ouders met secundair of hoger onderwijs. De analyse van biofysische factoren toonde aan dat hoger gelegen gebieden een hogere prevalentie vertonen, waarschijnlijk omdat in deze gebieden de toegankelijkheid van voedsel en gezondheidszorg beperkter is. De gebruikte proxy voor mycotoxinen expositie indiceerde dat meer perifeer gelegen gebieden met uitsluitend toegang tot een rurale markt geassocieerd zijn met een hogere prevalentie van groeiretardatie. Ook deze resultaten geven aan dat nader onderzoek naar mycotoxinen exposite via de voedselvoorzieningsketen in Rwanda noodzakelijk is.

De *vierde fase* van het onderzoek, eveneens op nationaal schaalniveau, richtte zich op het modelleren van de ruimtelijke variabiliteit van, en de risicofactoren voor, groeiretardatie in Rwanda op basis van een Bayesiaans geo-statistisch model. Het geo-statistische model toonde aan dat leeftijd en geslacht van het kind, geboortegewicht, exclusieve borstvoeding, diarree in twee weken voorafgaand aan het onderzoek, BMI van de moeder, opleidingsniveau van de moeder, welvaartsscore, vloertype van het huis, en toegang tot gezuiverd water significant samenhangen met groeiretardatie.

Vervolgens is geanalyseerd of de residuen van het geo-statistisch model ruimtelijke autocorrelatie vertoonden. Autocorrelatie zou betekenen dat er een of meer factoren zijn die bijdragen aan groeiretardatie maar ontbreken in het geo-statistisch model. Analyse van de residuen toonde aan dat deze inderdaad ruimtelijk gecorreleerd waren. Visualisatie van de residuen illustreerde het bestaan van gebieden met een onverklaard

hoog, en onverklaard laag, risico op groeiretardatie in een aantal regio's in Rwanda. Toekomstige beleidsinterventies om lineaire groeiachterstand in Rwanda te verminderen zullen rekening moeten houden met deze ruimtelijke heterogeniteit van groeiretardatie en moeten derhalve geografisch expliciet zijn. Ook is nader onderzoek nodig om de nog onbekende ruimtelijk expliciete factoren te identificeren die samenhangen met een verhoogd risico van achterblijvende lengtegroei.

In algemene zin heeft dit onderzoek bevestigd dat het voedsel van kinderen in het Musanze-district (te) weinig essentiële voedingsstoffen bevat, vooral micronutriënten zoals zink. Het onderzoek heeft ook aangetoond dat blootstelling aan aflatoxinen bijdraagt aan achterblijvende lengtegroei. Op nationaal niveau is de prevalentie ruimtelijk heterogeen waarbij met name de noordelijke en westelijke provincies gekenmerkt worden door een (zeer) hoge prevalentie. Het onderzoek heeft, naast voedselkwaliteit, belangrijke sociaaleconomische en geofysische factoren geïdentificeerd welke samenhangen met groeiretardatie. Tenslotte, heeft de geo-statistische analyse aangetoond dat achterblijvende lengtegroei een complex multifactorieel probleem is en dat nader onderzoek nodig is om de nog onbekende ruimtelijk expliciete factoren te identificeren die samenhangen met een verhoogd risico op achterblijvende lengtegroei in Rwanda.

Biography of the author

Vestine Uwiringiyimana was born on 20th July 1985 in Kampala, Uganda. In 2009, she graduated from the former Kigali Institute of Science and Technology, now College of Science and Technology of the University of Rwanda, where she obtained a Bachelor degree in Food Science and Technology with first-class honours. She worked as a tutorial assistant until 2010, when she went to pursue her MSc degree at Wageningen University and Research Centre, the Netherlands. She graduated in September 2012 with a Master of Science degree in Nutrition and Health and continued to work for the University of Rwanda as an assistant lecturer. During that time, Vestine was also a visiting lecturer in the Department of Human Nutrition and Dietetics of the College of Medicine and Health Sciences of the University of Rwanda. She was also involved in the HarvestPlus Challenge Program on the research on biofortified beans in Rwanda. In 2014, under the NICHE project funded by Nuffic, she obtained a scholarship to pursue her doctoral research at the Faculty of Geo-Information Science and Earth Observation of the University of Twente, the Netherlands. Her research outputs were presented and published in international and regional conferences and journals and resulted in this thesis.

Scientific publications (published and upcoming)

1. **Vestine Uwiringiyimana**, Marga C Ocké, Sherif Amer, Antonie Veldkamp (2018). Predictors of stunting with particular focus on complementary feeding practices: A cross-sectional study in the Northern Province of Rwanda. Nutrition. doi: 10.1016/j.nut.2018.07.016
2. **Uwiringiyimana, V**., Ocké, M. C., Amer, S., & Veldkamp, A. (2018). Data on child complementary feeding practices, nutrient intake and stunting in Musanze District, Rwanda. Data in Brief. doi: 10.1016/j.dib.2018.09.084
3. **Uwiringiyimana, V**., Amer, S., & Veldkamp, A. Stunting spatial pattern in Rwanda: an examination of the demographic, socio-economic and environmental determinants (Accepted for publication in Geo-Spatial Health)
4. **Uwiringiyimana, V.**, Osei Frank B., Amer, S., & Veldkamp, A. Stunting spatial pattern in Rwanda: Bayesian geostatistical modelling of stunting in Rwanda: risk factors and spatially explicit residual stunting burden (Submitted to BMC Public Health)

5. **Uwiringiyimana, V.**, Ocke, M. C., Amer, S., & Veldkamp, A. Exposure to aflatoxins from maize and peanut flours and stunting in young children from the Northern region of Rwanda (To be submitted to Food and Nutrition Bulletin)

Conference abstracts

1. **Vestine Uwiringiyiman**a, Marga C Ocké, Sherif Amer, Antonie Veldkamp (2018). Aflatoxin exposure among infants and young children in the Northern Province of Rwanda. World Mycotoxin Forum, Amsterdam-Netherlands 12-14 March 2018.
2. **Vestine Uwiringiyimana**, Marga C Ocké, Sherif Amer, Antonie Veldkamp (2017). Complementary feeding practices and stunting among infants and young children in the Northern Province of Rwanda. International Congress of Nutrition IUNS 12th ICN, Buenos Aires-Argentina, 15-20 October 2017.
3. **Vestine Uwiringiyimana**, Antonie Veldkamp, Marga C Ocké, Sherif Amer (2017). Dietary zinc intake, complementary feeding practices and stunting among children in the Northern Province of Rwanda. University of Rwanda Scientific Conference Week, Kigali-Rwanda, 14-16 June 2017.
4. **Vestine Uwiringiyimana**, Antonie Veldkamp, Marga Ocke, Sherif Amer (2016). Can low dietary zinc intake and mycotoxin exposure explain the stunting pattern in Rwandese children? International Conference on Geospatial Technologies for Sustainable Urban and Rural Development (GeoTechRwanda), Kigali-Rwanda, 18-20 November 2015.
8. **Vestine Uwiringiyimana**, Martin N. Mwangi, Jane Badham, (2015). Stakeholders and sustainability: Elements of an enabling environment. Micronutrient Forum Global Conference Proceedings, Sight and Life Magazine; pp. 75-81
9. Ongol, M.P., **Uwiringiyimana, V.**, Dusingizimana, T., Habimana, V., Owino, J., (2014). Retention of micro-minerals and degradation of anti-nutritional compounds during the traditional Rwandan bean cooking process. Micronutrient Forum 2014. Addis Ababa-Ethiopia, 2-6 June 2014.
10. Mukamushengezi, C., Iragena, A., Bagabo, G., Rusaro, J., **Uwiringiyimana, V**., Dusingizimana, T., Habimana, V., Joseph Owino, J., Ongol, M.P., (2014). Micro-mineral and anti-nutritional content of legumes and cereal-based foods commonly consumed in Rwanda. 3rd National Food and Nutrition Summit, Kigali-Rwanda, 18-20 February, 2014.