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Factors Associated with Anemia and Micronutrient Deficiencies in Children from Guatemala and Haiti

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Dedication

To my husband Matthew, my son Lucas and my daughter Nina.

A special mention to Dr. Richard Finnell who gave me the opportunity of a lifetime, to Dr. Jeanne Freeland-Graves and Dr. Gregory Reinhart for their unconditional support.

Factors Associated with Anemia and Micronutrient Deficiencies in Children from Guatemala and Haiti

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Anemia affects >2 billion people worldwide and is negatively associated with child development and academic performance. The purpose of this dissertation was to identify variables associated with anemia in children from Guatemala and Haiti.

Generalized linear mixed models were used to identify significant associations with anemia (dependent variable) and maternal, child and household variables utilizing a cross-sectional design. For the Guatemala study, serum biomarkers (soluble transferrin receptor, ferritin, zinc, folate, vitamin B12, C-reactive protein, and α1-acid glycoprotein) were explored. For the Haiti study, a mediation analysis was constructed to understand the relationship between rural or urban residence and anemia.

In Guatemala, 56% of children ages 6-24 months had anemia. For this age group, anemia was inversely associated with higher numbers of adults living in the household [OR=0.69; 95% CI (0.53-0.90)], and zinc deficiency increased the odds of anemia [OR=3.40; 95% CI (1.54-7.47)]. Conversely, only 12.1% of children ages 36-60 months had anemia, which was inversely associated with age [OR=0.90; 95%CI (0.81-1.00)].

In Haiti, 52.3% of children from rural areas had anemia, and stunting increased the odds of anemia [OR=3.42; 95%CI (1.47-7.97)], while children in households with more adults had lower odds, [OR=0.74; 95%CI (0.62-0.89)]. Among urban children, anemia prevalence was 75.4% and helminth morbidities increased the odds of anemia [OR=1.74; 95%CI (1.13-2.68)]. Age was inversely associated in both rural and urban children, [OR=0.88; 95%CI (0.81-0.96)] and [OR=0.93; 95%CI (0.88-1.00)], respectively. Only helminth morbidities partially mediated the relationship between anemia and place of residence (b=0.10, SE=0.05, P=0.037).

In conclusion, zinc deficiency was associated with anemia in young Guatemalan children, highlighting the need of continued multidisciplinary interventions with multiple micronutrients.

In Haitian school-aged children, anemia continues to be a severe public health problem. Key differences between urban and rural children were identified that should be considered when developing cost-effective interventions to improve nutritional and health status of this population, especially deworming measures in children residing in urban areas.

Further research examining how household composition, feeding practices, accessibility to micronutrient supplements, and decreasing intestinal morbidity is needed to develop effective interventions seeking to improve the nutritional status of children in Guatemala and Haiti.

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Chapter 1: Introduction and Review of the Literature

Undernutrition occurs when an individual has repeated infectious diseases and an insufficient intake of nutrients required for normal growth and developmental processes. More than 50% of the deaths in young children are attributed to undernutrition [1]. For children under 5 years of age [2], malnutrition contributes to the 21% of global disability adjusted-life years (DALYs).

Anemia is a common clinical manifestation of undernutrition and micronutrient deficiencies. It occurs when either the number of erythrocytes or their capacity to carry oxygen is insufficient to reach physiologic requirements that depend on age, sex, elevation, and smoking status [3]. Globally, 2.36 billion people are estimated to exhibit this condition, of which 273 million are children under 5 years of age [4].

According to a study by Vos *et al*., the leading cause of disability in children and adults under 39 years of age is iron-deficiency anemia [4]. It is well documented that anemia is multifactorial and influenced by genetic, environmental, and sociodemographic interactions [5]. Yet, more than half of anemia cases worldwide have been attributed to iron deficiency [4, 6]. However, findings from more recent analyses suggest that the contribution of iron deficiency in the etiology of anemia might be considerably lower. In countries where the prevalence of anemia is below 40%, iron deficiency may not cause more than 25% of anemia cases; in countries where the prevalence of anemia is above 40%, iron deficiency may cause only ~ 14% of anemia cases. [5]. Thus, other nutritional and non-nutritional causes may be critical in the etiology of anemia and further investigation is warranted.

Two large studies by the World Health Organization have estimated that the most susceptible age groups to experience anemia are children <5 years of age and pregnant women [7, 8]. The explanations for why young children are highly susceptible to anemia are diverse. Young children are vulnerable to nutritional insults during the first years of life, as growth rates are accelerated during these earliest ages. By two years, a child has increased their birthweight three-fold and almost doubled their stature [9]. Thus, micronutrient requirements are high, despite a limited gastric capacity. Furthermore, the complementary feeding period, (introduction of foods different than breastmilk) is a vulnerable period for the development of nutritional deficiencies. The World Health Organization (WHO) recommends that at 6 months of age, foods other than breastmilk should be introduced; as it is unlikely that breastmilk will continue to optimize requirements of the growing infant [10, 11]. If the complementary feeding practices are inappropriate or insufficient, the child is at high risk to become undernourished and more susceptible to disease [12].

GUATEMALA: UNDERNUTRITION AND ANEMIA

Guatemala is a multi-ethnic country in Central America, with an estimated population of ~16.5 million [13]. About 40% of its population is indigenous, predominantly of Mayan descent [14]. With 25 distinct ethnic groups, this Central American country is one of the most culturally diverse of the Americas. Unfortunately, Guatemala shows a modest development, despite having the largest economy in Central America. Poverty affects 59% of the population [15] and is concentrated in rural locations, in which 76% of people are poor. About 43% of the population is indigenous, and more likely to be living in poverty and exhibit disease and malnutrition, than those who are non-indigenous [16, 17]. Approximately half of the society practices agriculture as their main revenue source, even though it only accounts for less than 15% of the gross domestic product. Due to the lack of reliable sources of water, most families depend on rainfall for crop irrigation, further placing families at risk of acute food insecurity when droughts and floods ravage the country.

From the 1960's to 1996, Guatemala went through a Civil War that resulted in more than 200,000 deaths, and 40,000 to 50,000 disappearances; 83% of the victims were of Mayan origin [18]. Today, after more than two decades of the Civil War, inequality, discrimination and racism are still common and widespread. Reconstruction of the social fabric in some areas, that were highly impacted during the conflict, is still ongoing [14].

The nutritional profile of Guatemalan children is unique. Nearly 50% of children under five years of age exhibit chronic undernutrition [19] (length/height-for-age z-score <-2), also referred to as *stunting*. The prevalence of stunting is the highest reported in the Western Hemisphere [20]. Similar to anemia, chronic undernutrition is a common manifestation of persistent micronutrient deficiencies, and is considered a functional indicator of population-wide zinc deficiency [21].

A 2013 study in the Western Highlands of Guatemala identified several factors associated with stunting in young children. These include: being ages 13–18 months, birth at home, low maternal education, Mayan ethnicity, maternal short stature, and having ever received iron supplementation [22].

In addition to stunting, the national prevalence of anemia in children under 5 years is 25%. This condition impacts 27.4% of children from rural areas, compared to only 20.7% in urban centers [19]. In children from women who identified with an indigenous ethnic background, anemia prevalence was 26.9% vs. 23.7% of the non-indigenous [63].

Similarly, young children from Guatemala experience a high infectious disease burden. The 2014-2015 Maternal and Child Nutrition Survey in Guatemala reported that 41.9% of children sought treatment for diarrhea in the previous 2 weeks, and 50.4% for acute respiratory infections [19]. The same survey reported a prevalence of stunting (length or height-for-age z-score under -2 standard deviations) of 46.5% in children under 5 years of age [19].

At present, there are significant data gaps concerning the factors and micronutrient deficiencies associated with anemia in young children from rural areas in Guatemala.

Aim 1 is to characterize and identify the factors and micronutrient deficiencies associated with anemia in infants/toddlers and preschoolers from Guatemala. Results will be derived from cross-sectional analyses of baseline data collected as part of a previous randomized controlled trial. This investigation assessed the efficacy of a micronutrient intervention coupled to a responsive feeding and parenting program in young children from a rural area in Guatemala (*Clinical Trial Registry number: NCT02302729*).

HAITI: UNDERNUTRITION AND ANEMIA

Haiti is the poorest nation of the Western Hemisphere and the fourth most unequal country in the world, with a Gini index of 60.8. The Gini coefficient is a measurement used to compare income or wealth distribution in a county and is the most commonly used measurement of inequality. This country has a weak economy that is heavily dependent on foreign aid and **remittances** (money sent from abroad as a gift from friends or family members) [13]. More than half of the ~11 million Haitians are living below the poverty line. The vast majority of the population are descendants of African slaves brought by the French during the 18th Century [13, 23]. About 56.2% of the total population lives in urban areas, and the rate of urbanization is ~2.9% [13]. According to the World Bank, poverty and extreme poverty are more prevalent in rural areas in this country. Access to improved water and sanitation constitutes a major challenge for many Haitians, especially the poorest [24]. Lack of access to improved water and sanitation has been extensively associated with increased morbidity and mortality [25].

The prevalence of anemia in Haiti is among the highest in the Americas. It is considered a severe public health problem in children under 5 years of age (66.3%), and in women of reproductive age (49.0%) [3, 26]. For comparative purposes, the prevalence of anemia in children under 5 years of age in the Dominican Republic was lower (28.1%) in 2014 [27], and in school-aged children, ~18% in 2012[1] [28]. About a fifth (21.9%) of children under 5 years of age are stunted, and 3.7% of children are wasted (weight-for height z-score < -2 standard deviations below the median) [26].

A considerable body of literature worldwide documents that children residing in urban locations tend to have better nutritional and health status, as compared to those living in rural areas [29]. In contrast, a few studies from Haiti have described higher proportions of anemia among urban children and women versus those living in rural areas. Heidkamp *et al.* [30] found that urban children under 5 years-of-age had a significantly higher prevalence of anemia than age-matched children from rural areas (65% vs. 56%, respectively). In women of reproductive age, the same trend was observed. A total of 54% of women of urban residence had anemia, compared to the 43% of women living in rural locations [30]. The most recent Haitian Survey of Mortality, Morbidity and Service Utilization (2016-2017) reported that the proportion of woman with anemia residing in urban locations continued to be higher, at 52.4%, compared to women in rural areas (46.0%) [26]. Furthermore, Iannotti *et al.* observed a prevalence of anemia in school-aged children living in urban areas at 70.6% [31] and in rural areas, 51.1% [32].

Several reasons may explain this urban-rural differences in anemia in Haiti. First, children living in rural areas may have greater accessibility to seasonal micronutrient-rich produce (e.g. mangoes). In contrast, urban populations may instead consume low nutrient-

[1] The micronutrient survey for school-aged children used the threshold of hemoglobin concentrations <12.0g/dL for children ages 6 to 14 years-old.

dense cereal staples more frequently. Micronutrient-rich foods in rural regions may help improve the nutritional status of children from rural areas, reducing the prevalence of anemia [30]. In addition to greater accessibility of micronutrient-rich food in rural locations in developing countries, livestock ownership is a common practice [33]. Access to farm animals may lead to an increased intake of animal source foods (e.g. meat and eggs). This could be due to direct consumption from the household, or indirectly through income generated from production sales [34]. Other reasons are that poor sanitation, limited access to potable water, and poor hygiene [35] could increase infectious morbidity and anemia in highly populated urban areas, as compared to rural regions [36].

The third aim of this dissertation is to understand the differences in the prevalence of anemia between Haitian children living in rural vs. urban areas. The first objective is to characterize baseline factors associated with anemia in rural and urban school-aged children from Haiti. The second objective is to compare sociodemographic, dietary, nutrition, and health/morbidity factors between children from urban or rural residence. The third objective will be to identify a mediating pathway to clarify urban-rural differences in anemia prevalence in this population.

NUTRITIONAL CAUSES OF ANEMIA

Nutritional causes of anemia in children may be related to a variety of vitamin and mineral deficiencies [37, 38] including iron, folate, vitamin B12, zinc, ascorbic acid, vitamin D and zinc. All these micronutrients have all been documented to cause, or be associated with anemia [39]. In this dissertation, iron, folate, vitamin B12 and zinc were analyzed in a sample of young Guatemalan children.

Iron deficiency anemia appears to be the most prevalent type of nutritional anemia in the world. Iron associates with the four heme-groups that create the structure of hemoglobin. In the erythrocyte, this structural protein binds with oxygen and carbon dioxide allowing aerobic respiration and energy production. Iron is a component of other heme- and non-heme proteins and cytochromes involved in a variety of metabolic processes in the body [39]. The metabolism of iron is a complex process that includes participation of several other essential vitamins and minerals such as riboflavin, ascorbate, vitamin D, and copper [40].

In addition to erythropoiesis, iron is essential to several biologic functions including cellular respiration, energy production, and DNA synthesis. The association of iron metabolism with other essential nutrients may explain why, in some cases, interventions focused solely on the provision of exogenous iron have had only a modest impact on the improvement of this condition [41].

The measurement of iron status in individuals is a challenge. Because iron is essential to both humans and pathogens, one of the non-specific mechanisms of acute phase response to infection or trauma is to limit availability of host iron to pathogens [42]. Ferritin is an intracellular protein that stores and releases iron and reflects iron stores in the liver [43]. Ferritin is also an acute phase response protein, and plasma concentrations of this molecule increase with inflammation [44]. Thus, the use of ferritin concentrations to assess iron status in individuals must be in consideration of inflammatory status [45].

Transferrin is a transport protein that associates with cellular transferrin receptors and delivers iron to the cells. A soluble form of the transferrin receptor has been identified. The soluble transferrin receptor reflects active erythropoiesis and early stages of iron deficiency [46]. Although it is relatively easy to measure, this receptor also is influenced by inflammation [47].

Folates and vitamin B12 (cobalamin), are other key nutrients involved in erythropoiesis [48]. These B vitamins act as coenzymes in nucleic- and amino- acid production, and exhibit an essential role in the conversion of homocysteine to methionine [48]. This step is key for methylation reactions and for the synthesis of s-adenosylmethionine, a primary methylating agent. These biochemical pathways are involved in gene expression regulation and protein structure, and play an important role in the generation of erythrocytes [48, 49]. In contrast with iron, folate and vitamin B12 deficiency result in megaloblastic anemia [50], as opposed to microcytic, hypochromic anemia.

The 2016 micronutrient survey in Guatemala reported a prevalence of serum folate deficiency below 2% in children < 5 years [51]. This suggests that folate deficiency may not be a significant factor associated with anemia in young Guatemalan children. In contrast, vitamin B12 deficiency was observed in ~22.5% of young children in the same survey [51], suggesting that this micronutrient plays a more important role in the development of anemia in this population. Regardless, the role of folate and vitamin B12 in the context of anemia in young Guatemalan children has not been analyzed.

Zinc is an important component of several transcription factors and enzymes implicated in multiple metabolic processes including DNA, RNA, and heme synthesis. All of these are needed for erythropoiesis [52, 53]. Zinc is thought to act as a stabilizing agent in red blood cell membranes [54], and as a modulator of erythroid gene expression [55]. These activities are critical in the maintenance of sufficient and functional mature erythrocytes required for an adequate oxygenation of tissues and organs. Approximately half of the children in Guatemala are stunted [19], and stunting is a functional indicator of population zinc deficiency [21]. This is critical to consider in the context of anemia in young children from Guatemala.

NON-NUTRITIONAL CAUSES OF ANEMIA

Anemia of Inflammation

Chronic diseases and infections are important contributors of the non-nutritional causes of anemia. “Anemia of inflammation” is a condition characterized by low plasma iron concentrations with preserved intracellular iron storages. It occurs in the context of systemic inflammation; chronic inflammation has been associated with decreased erythropoiesis [56].

In contrast, iron-deficiency anemia occurs when both plasma and intracellular concentrations of iron are depleted. In populations with a high infectious burden and where micronutrient deficiencies are prevalent, iron deficiency anemia and anemia of inflammation often coexist [57]. For instance, a study that included 16 surveys from countries within Latin America, Asia and Africa, reported that 55% and 58% of preschool-aged children had concomitant iron deficiency, in association with high and very high infection categories, respectively [38].

In 2019, Ganz [57] provided an excellent review on the anemia of inflammation. This condition occurs when proinflammatory cytokines are activated during systemic inflammation. Besides other systemic functions associated with immunological responses, activated cytokines favor myeloid synthesis (precursors of leukocytes), as opposed to erythroid precursors, decreasing the synthesis of red blood cells. Cytokines also activate macrophages for erythrophagocytosis, macrophage activation results in a shorter lifespan of erythrocytes [57]. Furthermore, interleukin-6 via hepcidin, inhibits the release of iron from macrophages, favoring systemic hypoferremia. Hypoferremia on its own reduces the proliferation of erythroblasts by downregulating the expression of erythropoietin receptors in erythroid progenitors, explaining the pathophysiology of anemia of inflammation [57].

Other conditions such as reduced kidney function can increase systemic concentrations of hepcidin; thus, resulting in macrophage sequestration of iron and inducing plasmatic hypoferremia [57].

In sum, erythropoiesis includes a combination of intricate metabolic processes, in which several micronutrients are involved. Thus, in geographic locations where micronutrient deficiencies and infections are common, micronutrients other than iron must be considered to better understand the etiology of anemia.

Parasitism

In the developing world, parasite infections such as malaria, hookworm and schistosomiasis are common and these may contribute to the development of anemia [4]. The Global Burden of Disease Study estimated that malaria affects ~351.1 million individuals, and about 80.6 million cases of anemia worldwide are related to malaria. The same study estimated that about 500 million people have hookworm infections [4]. In 2013, about 55.2 million cases of anemia worldwide were reported to be due to hookworm and schistosomiasis infections [4]. Malaria is an infectious disease that results from the infection of six species of parasites from the genus *Plasmodium.* These parasites are transmitted through the bite of mosquitoes from the genus *Anopheles*. The most virulent species of the *Plasmodium* parasite is the *falciparum*, as most deaths and severe diseases are principally caused by this microorganism [58]. Malaria causes hemolysis of infected and healthy erythrocytes, as well as decreased erythropoiesis, thereby slowing the physiologic response to the injury [58]. Haiti is an endemic region for malaria, and shows the highest number of malaria cases of the Caribbean and Central America with an annual incidence of 1,278 per 100,000 people [59].

Other parasites involved in the etiology of anemia are prevalent in Haiti. A 2002 study in Haitian school-aged children identified a 45.8% prevalence of intestinal helminthiasis in the Nord department, with a 28% rate of two or more helminths. The most prevalent helminth is *Ascaris lumbricoides* (27.3%), followed by *Trichuris trichiura* (7.3%) and *Necator americanus* (hookworm) (3.8%) [60]. The latter is the most commonly associated with anemia, as it attaches to the intestinal wall and feeds directly from the host's blood [61].

Amebiasis and giardiasis are intestinal protozoan parasites that are frequent in Haiti. These are the leading causes of diarrhea and dysentery (diarrhea with blood and mucus) worldwide [62]. The global burden of amebiasis is difficult to establish; however, protozoan parasites are endemic and ubiquitous in Central and South America, Africa, and Asia [62]. The cysts of *Entamoeba histolytica* are environmentally stable and only require a few cysts to generate infection. Furthermore, the cysts are resistant to chlorine, which makes these difficult to kill [63]. The mechanism of anemia of *E. histolytica* infection is due to blood loss and chronic inflammation of the colonic mucosa [64]. Giardiasis generates intestinal malabsorption in addition to intestinal mucosal inflammation, thus contributing to anemia by decreased nutrient absorption and diarrhea [65]. A recent meta-analysis by Donohue et al., 2019 found that co-infection with two or more species of helminths, or helminth- intestinal protozoan were significantly more common than single infections. Additionally, individuals with polyparasitism had significantly lower hemoglobin levels and higher anemia prevalences [66].

Consequently, children with intestinal parasites are likely to be infected with other species of intestinal parasites that might be contributing to the anemia burden. In this dissertation, intestinal worm morbidities were recalled, and fecal samples were not collected. Thus, this analysis did not include a characterization of intestinal parasites.

Anemia of Hereditary Origin

A number of hereditary disorders are known to cause anemia. The most common ones include glucose-6-phosphate dehydrogenase deficiency, sickle cell disease, and thalassemia [67]. All these genetic conditions occur in high rates in African populations [68-70].

Haitians may exhibit similar prevalences of hemoglobinopathies as do populations from Sub-Saharan Africa [23, 71]. One limitation to the research reported in this dissertation is that these genetic variants in the Haitian children were not explored.

Other Dietary and Sociodemographic Factors Associated with Anemia

A number of dietary and sociodemographic factors have been reported to be associated with anemia in children. These include dietary, socioeconomic, (poverty, maternal education, place of residence, belonging to an ethnic minority, maternal employment and marital status), poor sanitation and lack of access to fresh water, and household structure.

Dietary factors that have been associated with anemia and undernutrition in children include inappropriate complementary feeding practices such as early introduction (before 6 months of age) of liquids other than breastmilk, infrequent consumption of animal source foods or micronutrient supplements, and monotonous diets [38, 72]. A study in Guatemala documented that infants under six months of age fed with watery juices -" "*aguitas*" were associated with stunting [73]. Conversely, consumption of fortified foods or supplements with iron, vitamin A, and other micronutrients have improved hemoglobin concentrations and anemia [74].

Socioeconomic status greatly influences the health and nutritional status of women and children [75]. Poverty increases household food insecurity [76], and thus the probability of exhibiting malnutrition and anemia among women and children [77, 78].

Education has been used as a proxy to determine family socioeconomic status [79]. Low maternal education has been associated with reduced health knowledge and use of health services; this often results in poorer nutritional and health status in the children [80].

Place of residence is another factor that has been reported to influence the health status of children. Urban living, in general, has been associated with better nutritional and health outcomes [81-83]. However in some countries, poor urban children have been found to have higher rates of stunting and mortality than their rural counterparts after controlling for sociodemographic factors [29]. In Guatemala, indigenous and rural populations tend to be the most unfavored, having received the least education, and develop widespread anemia and malnutrition [19, 84]. Other studies have documented that Hispanic and African American minority groups in the U.S. have a higher odds of adverse health and nutritional outcomes [85, 86].

Maternal employment and single parenting also have been associated with the health status of a child. Worldwide, maternal employment coincides with a decreasing prevalence of undernutrition among women and children (57). Whether maternal employment is a risk for undernutrition is contradictory (56, 58, 59). In some cases, children living in uniparental households (principally mothers) have shown an increased probability of poor health and malnutrition (60-62), especially in situations of poverty. The effect of maternal employment and solo parenting status on the health of a child is often ameliorated as socioeconomic status increases. In addition, it can be largely mediated by other environmental factors (60). A qualitative analysis in a small subsample of mothers from children participating in this trial identified that the principal motivation of mothers to seek employment outside the home was the need for income. Employment could lead to better food purchases and overall household well-being as more income could be spent on children. However, employment could impact maternal time allocation, especially decreased time spent on meal preparation, food procurement, and childcare (63).

Larger homes, especially those with more children, also have been associated with anemia and other adverse health and nutritional outcomes [87, 88]. Other conditions commonly coexisting with anemia and poverty include low birthweight, stunting, and wasting [31, 89], and maternal anemia [90].

Poor sanitation and lack of access to potable water is common in households with adverse socioeconomic conditions. Lack of access to safe water and sanitation increase infectious disease burden, and as a result, anemia [35]. Rural areas of Latin America and the Caribbean, including Haiti and Guatemala, tend to have lower access to improved water and sanitation [24]. This situation is exacerbated by the fact that many of these locations are endemic for vector-transmitted diseases that cause anemia, such as dengue, chikungunya, yellow fever, and malaria. And for many households without tap water, their water reservoir may be favoring breeding of these mosquito vectors [91].

In conclusion, anemia is a complex condition that is a significant public health problem in numerous regions of the world, especially in countries with developing economies. The genetic, nutritional, infectious and environmental factors that contribute to anemia largely depend on the geography and population characteristics. Thus, it is important to examine each population individually. In the context of Guatemala, with one of every two children exhibiting stunting, factors beyond iron deficiency may be playing significant roles. In Haiti, where the infectious burden is high, non-nutritional causes may have a more meaningful role than previously thought.

Chapter 2: Zinc Deficiency Associated with Anemia among Young Children in Guatemala

ABSTRACT

One in four children under age five in Guatemala experience anemia (hemoglobin <11.0 g/dl). This study characterized the factors and micronutrient deficiencies associated with anemia in a baseline cross-sectional sample of 182 Guatemalan infants/toddlers and 207 preschoolers, using generalized linear mixed models. Associations between anemia and maternal, child and household variables, and biomarkers: soluble transferrin receptor, ferritin, zinc, folate, vitamin B12, C-reactive protein, and α1-acid glycoprotein were explored.

Rates of anemia were 56% among infants/toddlers and 12.1% among preschoolers. In children with anemia, rates of iron deficiency (low ferritin based on inflammation status, and/or high soluble transferrin receptor, ≥1.97 mg/L) and zinc deficiency (serum zinc <65 μg/dL) were 81.1% and 53.7% respectively. Folate deficiency (either plasma folate <3 ng/mL, or erythrocyte folate <100 ng/mL) was 3.3%. Vitamin B12 deficiency (plasma vitamin B12 <148 pmol/L) was 7.5%. For infants and preschoolers (<24 months), the odds ratio of anemia was lower when higher number of adults lived in the household [OR=0.69; 95% CI (0.53-0.90)], and higher when children were zinc deficient [OR=3.40; 95% CI (1.54-7.47)]. For preschoolers (36-60 months), the odds ratio of anemia was lower for every additional month-of-age [OR=0.90; 95% CI (0.81-1.00)]. Findings suggest that micronutrient deficiencies coexist in Guatemalan rural children, and zinc deficiency is associated with anemia in children <24 mo, highlighting the need of continued multidisciplinary interventions with multiple micronutrients. Further research examining how household composition, feeding practices, accessibility to micronutrient supplements

and to animal source foods is needed to incorporate strategies to improve the nutritional status of Guatemalan children.

INTRODUCTION

Anemia is a widespread public health problem that affects more than 2 billion people worldwide [4]. Anemia is negatively associated with children's physical and cognitive development, behavior, and academic performance [92-94].

Anemia has been associated with iron deficiency and other disadvantages associated with poverty. Recent meta-analyses and systematic reviews have found mixed findings related to improvement in childhood development after up to six months (mo) of iron supplementation/therapy and resolution of anemia, suggesting that other factors associated with anemia should be examined [95]. Furthermore, children with anemia or undernutrition have higher morbidity and mortality rates, worse academic performance, and lower earning capacity as adults [96-98].

In Guatemala, the latest maternal and child survey in 2014-2015 reported a national anemia prevalence of 25% among children under 5 years of age [19], a decline from 47.7% in 2008–2009 [99].

Anemia continues to be an important public health problem, especially among children aged 6 to 11 mo, with a prevalence of 64.3%, and those living in rural areas and of indigenous ethnicity seem to be the most vulnerable [19].

While anemia in Guatemala is poorly characterized, multiple factors have been recognized that are associated with stunting in the region, which is widely prevalent in children <5 years at 46.5% [19]. Some of the factors associated with stunting in the region include being ages 13–18 mo, born at home, Mayan (indigenous) ethnicity, low level of maternal education, short stature, and consumption of iron supplementation [84]. The high prevalence of stunting in this country suggests zinc deficiency in children might be widespread [21]. This is supported by the 2008-2009 national Micronutrient Survey that reported a national prevalence of zinc deficiency of 34.9% [100]. In the same survey, the prevalence of children with low ferritin (<12 μg/L) and no inflammation (AGP <1 g/L) was of 18.6%.

Currently, little is known to what extent anemia in young children of rural origin in Southwestern Guatemala is associated with micronutrient deficiencies or other socio-cultural factors. Yet, the rates of anemia and stunting of children <5 years of the Southwestern region in Guatemala are higher than national average at 31.3% and 51.9%, respectively [19].

The purpose of this study was to characterize the factors and micronutrient deficiencies associated with anemia in a population of infants/toddlers and young children and preschool-aged rural children from Southwest Guatemala through a cross-sectional secondary analysis of baseline data from a randomized controlled trial.

METHODS

Location

The study was conducted in the Municipality of Nuevo San Carlos, in the Department of Retalhuleu, Guatemala. Baseline data were collected between March and May of 2015. According to the National Statistics Institute of Guatemala, Retalhuleu has a population of ~300,000, of which 84.3% is non-indigenous [19].

Design of Study and Participants

This cross-sectional secondary analysis used baseline data collected in a randomized controlled trial. The original trial was designed to examine the effect of a micronutrient supplement and an early learning intervention on the growth, development and health of infants and toddlers ages 6-24 months and preschoolers from 36-48 months with length/height-for-age z-score < -1, a marker of vulnerability. Children younger than 6 months were not recruited to avoid interfering exclusive breastfeeding with the nutrition supplement. Inclusion criteria for parents or guardians were age ≥18 years, Spanish speaker, resident in the study community, and not planning to leave the region in the next year. Exclusion criteria for children were severe stunting [length or height-for-age z-score <-3 SD], severe anemia, [hemoglobin (Hb) <7 g/dL)], product of a multiple pregnancy, genetic or chronic conditions or disability. Children with severe stunting and anemia were referred to local resources for further evaluation and treatment as indicated. Mothers of participants were informed in advance about the specific date, time and location of the recruitment and data collection sessions. Data collection was conducted in a community center with transportation provided.

Based on financial limitations, blood biomarkers were collected from a subsample of 412 participants (189 infants/toddlers, 212 preschoolers), selected through a randomization procedure. Eleven participants did not complete the enrollment survey (four infants/toddlers, seven preschoolers) due to different reasons, nine had severe stunting (five infants/toddlers, four preschoolers), two had severe anemia (one infant, one preschoolers), and one infant had both severe anemia and severe stunting. The resulting analysis sample included 182 infants/toddlers and 207 preschoolers.

The sample size for the clinical trial was determined by the expected effect size of the intervention (multiple micronutrient fortification powder) on growth and development. Because the cross-sectional sample size was fixed, and no prior studies have shown "true" effect sizes for zinc deficiency and anemia in a similar population. Thus, post-hoc power calculation was not performed using the effect size observed in the analysis, as recommended [101].

Data collection

A baseline survey that included family demographics, social and environmental factors was adapted from previously validated questionnaires and administered to mothers/caregivers at the community center by local trained field workers [102].

Anthropometry

Anthropometry was collected by a standardized team following a detailed protocol [103].

Weight was measured using the Health-o-meter 553KL Digital Portable Pediatric Baby Scale® (Pelstar LLC, Countryside, IL, USA) for infants/toddlers and the digital weight scale Tanita, UM-028® (Tanita, Chicago, IL, USA) for preschoolers. Recumbent length was measured for infants/toddlers; standing height was measured for preschoolers using Shorr Board® stadiometers (Weigh and Measure LLC, Olney, MD, USA). Anthropometric z-scores were calculated using the 2006 WHO Child Growth Standards.

Maternal, Socioeconomic and Home Environment Variables

To determine household economic resources, a questionnaire listing 28 household items adapted from the 2014 Guatemala National Survey of Living Conditions was implemented [104]. An assets variable was created by adding all positive answers with higher scores indicating more assets.

Home environment measurements including child care indicators adapted from the Home Observation for Measurement of the Environment Inventories (HOME) [105] and The Household Chaos Scale [106] were collected at baseline and used in the analysis.

Maternal employment was defined as mothers working for four or more days per week outside the home.

Diversity of Diet

A semi-quantitative food frequency questionnaire based on commonly consumed foods during the previous week was adapted and implemented [107]. Dietary data were computed into seven food groups (grains, roots and tubers, legumes and nuts, milk and dairy products, meat, fish, poultry and organ meats, eggs, vitamin A-rich fruits and vegetables, and other fruits and vegetables). Each food group was scored as '0' if there was no consumption reported, or as '1' if consumption of at least one food item within that food group was reported. The weekly dietary diversity score for each participant was computed as the sum of the number of positive answers for the food groups consumed in the previous seven days. Higher scores represented a more diverse diet.

Samples and Analysis of Blood

Fasting venous blood was drawn from the antecubital vein with stainless-steel needles by a trained phlebotomist in a closed room. Whole blood was collected into trace element-free, polyethylene tubes. Samples were refrigerated at -8◦C immediately after extraction and shipped to the laboratory for further processing the day of collection, following the INCAP laboratory protocol of sample shipping and handling and the procedures recommended by the International Zinc Nutrition Consultative Group [108]. Ferritin, folate and vitamin B12 were measured using a chemiluminescence immunoassay method (CLIA), with the Maglumi 1000 (SNIBE, Shenzen, China PR). CRP and AGP1 were detected using a nephelometer (BN ProSpec, Dade Behring, Germany). Hb was measured using whole blood on an automated hematology analyzer (Hemaray 83 Rayto, Shenzhen, China PR) and sTfR was determined via an in-house ELISA [109]. Serum zinc was assessed by microwave plasma atomic emission spectrometry (MP-AES 4200, Agilent Technologies, Australia).

Anemia was defined per World Health Organization standards as Hb <11 g/dl [3].

Subclinical inflammation was defined as CRP >5 mg/L and/or AGP >1 g/L. Inflammation status categories were defined using the above CRP and AGP concentrations: 1) reference (normal CRP and AGP); 2) incubation (elevated CRP and normal AGP); 3) early convalescence (elevated CRP and AGP); and 4) late convalescence (normal CRP and elevated AGP).

Normality of biomarker concentrations was visually inspected using frequency distributions (histograms) and considered normal if the ratios of skewness to its standard error, and kurtosis to its standard error were between ±2. Variables that did not follow a normal distribution were log-transformed.

Corrected values of ferritin, sTfR and zinc within inflammation groups were calculated by multiplying individual concentrations by their group correction factors. For ferritin, the correction factors were 0.77 (incubation), 0.53 (early convalescence) and 0.75 (late convalescence) [45]. For sTfR and zinc, the correction factors were the ratios of the respective geometric means of each biomarker concentrations of the 'reference' group to the respective inflammatory group. For sTfR, the natural-log transformed concentrations were multiplied by 1.40 (incubation), 1.23 (early convalescence), and 0.94 (late convalescence) [47]. For zinc, the natural-log transformed concentrations were multiplied by 0.998 (incubation), 1.022 (early convalescence), and 0.990 (late convalescence) [110]. To facilitate interpretation, the results were converted back to the original scale. Cutoffs for ferritin were determined based on inflammation status as follows: reference, <12 mg/L; incubation and early convalescence <15 mg/L; and late convalescence <22 mg/L. The cutoff used to define elevated sTfR was determined using the geometric mean of children with no subclinical inflammation, no anemia, and normal ferritin concentrations (1.97 mg/L) [111]. Iron deficiency (ID) was defined as either low ferritin and/or high sTfR. The cutoff used to define zinc deficiency was of <65 µg/dL. Folates were analyzed using both hematologic indicators for defining folate deficiency (either plasma folate <3 ng/mL, or erythrocyte folate <100 ng/mL) (WHO, 2012). Vitamin B12 (VB12) deficiency was defined as <148 pmol/L (200 pg/mL) [112].

Analysis of Statistics

Data were entered into Magpi® (Magpi, Washington, DC, USA) a cloud-based software, and the database was exported to SPSS® statistical software package version 24.0 (IBM SPSS Inc., Chicago, IL, USA) for analysis.

Infants/toddlers were analyzed separately from preschoolers because anemia rates, diets, nutritional status, maternal education and maternal employment status differed between the two samples. To identify maternal, child and household factors associated with anemia, a literature review was performed to identify potential variables associated with anemia. Those variables available from the survey, previously identified in the literature review where included in a univariate analysis using logistic regressions. At this stage, the same variables were included in both age categories. To increase the precision of the final models, a forward selection method was used to develop a prediction logistic model using the variables identified in the previous step with a p-value ≤0.2 and incorporated into a logistic regression, using a generalized linear mixed model (GLMM). A final step included micronutrient deficiencies as fixed effects with a random intercept for cluster. Final GLMMs were adjusted for maternal education, age, and sex, even though age and sex were not significant in the bivariate analysis. Given the low prevalence of folate and vitamin

B12 deficiencies, these were not included in the final models; however, the final models were adjusted by the continuous concentrations of folate and vitamin B12.

To further investigate the association of the sociodemographic variables, in particular “number of adults living at home” with anemia in the final models, a Poisson model was created using the significant variable as dependent and childcare- and other sociodemographic indicators as independent variables. The dependent variable was also categorized to identify how many adults would be needed in the household to reduce the odds of anemia.

Sensitivity analyses included the construction of unadjusted models with individual micronutrient deficiencies as fixed effects.

RESULTS

Background Characteristics

Baseline characteristics of infants/toddlers and preschoolers are listed in Table 1. Children were evenly distributed by sex in both age categories. Mean age was 13 mo for infants/toddlers and 45 mo for preschoolers. Eighty percent of infants/toddlers and 3.9% of preschoolers were breastfeeding. Introduction of complementary foods occurred around the fifth mo of age in both age categories. Weekly dietary diversity scores were lower in infants/toddlers vs. preschoolers (5.6 vs. 6.1, $p<0.001$). Consumption of meat, fish, poultry, or organ meats at least once during the previous week by age category were infants <12 mo=47.1%; toddlers 12-21 mo= 87.5%; preschoolers 36-<48 mo =95.5%; and preschoolers >48 to <60 mo = 95.8%. The prevalence of stunting and underweight were both significantly lower among infants/toddlers than preschoolers (25.8% vs. 41.1%, $p=0.002$, and 3.3% vs. 13.0%, $p<0.001$, respectively). The prevalence of wasting was below 3.5% in both groups.

A larger proportion of mothers in the infants/toddlers group had completed primary school compared to mothers of preschoolers (30.2% vs. 21.4%, p=0.048).

Overall, more mothers of preschoolers were overweight or obese, compared to mothers of infants/toddlers (43.4% for mothers of infants/toddlers, and 59.4% for mothers of preschoolers, p=0.002). More than 50% of the households reported food insecurity, with a higher rate in mothers of preschoolers (55.2% in infants/toddlers and 65.2% in preschoolers, p=0.045). Mothers of preschoolers were twice as likely to be employed outside the home compared to mothers of infants/toddlers, (6.6% vs 13.5%, p=0.025). About 15% of mothers from both infants/toddlers and preschoolers were single, widowed, or divorced. Mean household income of 1,257 GTQ/mo (equivalent to 164.3 USD/mo; exchange rate of March 30, 2015) was similar in both groups.

Description of anemia, micronutrient and inflammation status

The overall prevalence across all age categories of iron and zinc deficiencies were 73.1%; 95% CI (68.0-78.2), and 36.3%; 95% CI (31.2-41.4), respectively.

Table 1: Demographic characteristics of infants and toddlers ages 6 to 24 months and preschoolers ages 36 to 60 months from Retalhuleu, Guatemala[1,2].

	Infants /toddlers n=182	**Pre-schoolers n=207**	**All n=389**
	Value	**Value**	**Value**
Age, mo	13.2±4.3	45.3±5.6	30.3±16.8
Female sex, %	55.5	46.4	50.6
Institutional birth, %	81.3	82.6	82.0
Prenatal checkups	4.3±3.0	4.9±3.2	4.6±3.1
Breastfeeding duration, mo	11.9±4.7	19.9±9.8	16.1±8.8
Age of introduction to complementary foods, mo	5.1±3.0	5.0±3.6	5.0±3.3
Weekly dietary diversity score[3]	5.6 ±1.5	6.1±0.9	5.9±1.3
Weekly consumption of meat, fish, poultry and organ meats, %	70.6	95.6	83.9
Stunted, %	25.8	41.1	33.9
Underweight, %	3.3	13	8.5
Wasted, %	3.3	2.4	2.8
BMI-for-age z-score >2 SD, %	1.7	2.4	2.1
Maternal age, yr	26.8 ±8.1	30.5±7.9	28.8 ±8.2
Maternal education (secondary or more), %	30.2	21.4	25.5
Maternal BMI ≥25.0, %	43.4	59.4	51.9
Household Food Insecurity Access Scale[4], %	55.2	65.2	60.6
Indigenous ethnicity, %	12.7	18	15.5
Number of <18 years old living in household	2.9 ±1.6	3.3±1.6	3.1±1.6
Number of adults living in household	3.1 ±1.6	3.0±1.4	3.0±1.5
Single, divorced or widowed, %	14.8	14.5	14.7
Maternal employment, %	6.6	13.5	10.3
Monthly household income, GTQ[5]	1233.8 ±698.5	1277.2 ±819.2	1257.0 ±764.7

[1] Values are means ± SDs unless otherwise indicated
[2] Groups significantly different by t- or chi-square tests
[3] FAO/FANTA Household Dietary Diversity Questionnaire and Guidelines
[4] FANTA Household Food Insecurity Access Scale (HFIAS) for Measurement of Household Food Access: Indicator Guide (V.3)
[5] GTQ, Guatemalan Quetzals (1GTQ = 7.65 USD using exchange rate of March 30, 2015).

Table 2 provides summary data on the prevalence of micronutrient deficiencies in children with anemia. More than half of the infants/toddlers had anemia [56%; 95%CI (48.8-63.3)], and experienced high rates of iron- and zinc- deficiency anemia, 82.9%; 95% CI (73.8-92.0) and 62.8%; 95% CI (52.4-73.2), respectively.

Table 2: Prevalence of micronutrient deficiencies and inflammation status in infants and toddlers ages 6 to 24 mo and preschoolers ages 36-60 mo with anemia[1].

	Infants/Toddlers		**Preschoolers**		**All**	
		95% CI	**%**	**95% CI**	**%**	**95% CI**
Anemia (Hb <11.0 g/dL)	56.0	48.8-63.3	12.1	7.6-16.6	127	28.0-37.3
Low ferritin[2]	53.8	42.5-65.2	14.3	2.0-30.6	45	35.5-55.4
Elevated sTfR (≥1.97 mg/L)	63.2	52.1-74.3	72.7	52.5-92.9	64	55.7-74.9
Iron deficiency (low ferritin and/or elevated sTfR)	82.9	73.8-92.0	75.0	54.2-96.0	73	72.9-89.4
Zinc deficiency (<65 µg/dL)	62.8	52.4-73.2	18.2	0.7-35.7	58	44.2-63.3
Folate deficiency[3]	3.0	-0.4-6.5	4.3	-4.7-13.4	4	0.1-6.5
Vitamin B12 deficiency (<200 pg/mL)	9.3	3.4-15.2	0	-	9	2.7-12.3
Elevated CRP (>5 mg/L)	13.6	6.3-21.0	20.8	3.3-38.4	17	8.4-21.9
Elevated AGP (>1.0 g/L)	41.1	30.8-51.5	34.8	13.7-55.8	45.0	30.7-49.0

[1] AGP, Alpha-1-acid glycoprotein; sTfR, soluble transferrin receptor; ID, iron deficiency; CRP, C-reactive protein

[2] Low ferritin was determined based on inflammation status as follows: Reference, <12 mg/L; incubation and early convalescence <15 mg/L; and late convalescence <22 mg/L.

[3] Either plasma folate <3 ng/mL, or erythrocyte folate <100ng/mL

The proportion of ID in infants/toddlers and preschoolers with no anemia was high, 80.3%; 95% CI (70.0-90.1) and 65.0%; 95% CI (57.1-73.0), respectively. Also, infants/toddlers and preschoolers with no anemia had a prevalence of zinc deficiency of 36.5%; 95% CI (25.2%-47.7) and 24.8%; 95% CI (18.2-31.5), respectively.

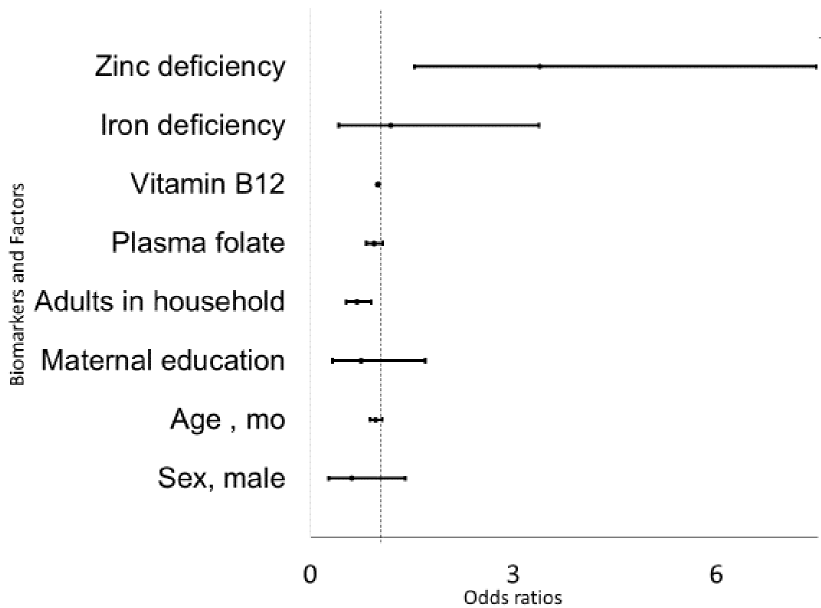
Rates of folate and vitamin B12 deficiency were under 10.0% in infants/toddlers and preschoolers. The inflammation status of infants/toddlers was 60.9% with no active inflammatory process (reference group), 1.2% in the incubation period, 9.9% in early convalescence, and 28% in late convalescence.

Among preschoolers, anemia prevalence was 12.1%; 95% CI (7.6-16.6) Three-fourths of preschoolers experienced ID, 18.2%; 95% CI (0.7-35.7) had zinc deficiency, 4.3%; 95%CI (-4.7-13.4) had folate deficiency, and none had vitamin B12 deficiency. The inflammation status of preschoolers was 58.9% with no active inflammatory process (reference group), none in the incubation period, 14.2% in early convalescence, and 26.8% in late convalescence.

Logistic Regression Analyses Associated with Anemia

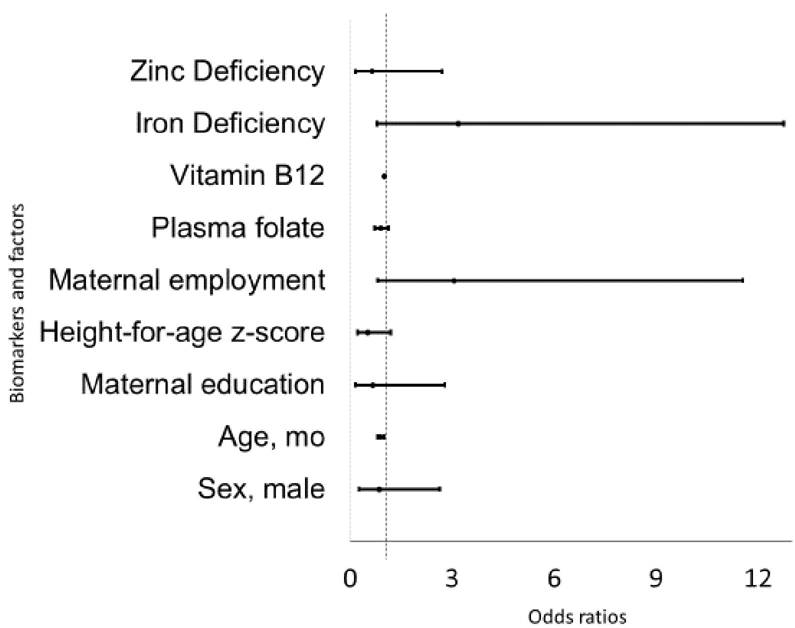
Figures 1 and 2 show the results from the multivariate logistic models testing the association between anemia and both blood biomarkers and significant maternal, child, and household factors in infants/toddlers and preschoolers, respectively.

Figure 1: Biomarkers and factors associated with anemia in infants and toddlers ages 6 to 24 months from Retalhuleu, Guatemala.



	OR	95% CI	P
Zinc deficiency	**3.39**	**1.54-7.47**	**0.003**
Iron deficiency	1.19	0.42-3.38	0.738
Vitamin B12	1.00	1.00-1.00	1.000
Plasma folate	0.94	0.83-1.07	0.362
Adults in household	**0.69**	**0.53-0.90**	**0.007**
Maternal education	0.75	0.33-1.70	0.487
Age , mo	0.96	0.88-1.06	0.427
Sex, male	0.62	0.27-1.40	0.245

Figure 2: Biomarkers and factors associated with anemia in preschoolers ages 36 to 60 months from Retalhuleu, Guatemala.



	OR	95% CI	P
Zinc Deficiency	0.64	0.15-2.70	0.542
Iron Deficiency	3.18	0.79-12.76	0.103
Vitamin B12	1.00	1.00-1.01	0.216
Plasma folate	0.91	0.72-1.14	0.414
Maternal employment	3.06	0.81-11.55	0.098
Height-for-age z-score	0.51	0.22-1.19	0.118
Maternal education	0.67	0.16-2.78	0.579
Age, mo	**0.90**	**0.81-1.00**	**0.045**
Sex, male	0.85	0.27-2.63	0.774

Zinc deficiency was associated with 3.4 times increased odds of anemia [OR=3.40; 95%CI (1.54-7.47). Child sex, age, maternal education, and ID were not associated with anemia. Separate models with either iron or zinc deficiency did not show major variability from the reported results. Among infants, the number of adults living at home and zinc deficiency were associated with anemia. For increasing numbers of adults living at home, the odds of anemia fell by 31% [OR=0.69; 95%CI (0.53-0.90)]. The significant association with anemia was observed when ≥4 adults lived on the household [OR=0.38; 95%CI (0.16-0.88].

To understand why increasing number of adults living at home was associated with lower odds of anemia in the infants/toddler group, further Poisson model analysis using "total count of adults living at home" (range=8) as the dependent variable, identified that households with more adults have higher assets scores: [OR=1.17; 95%CI (0.06-0.25)]. Childcare indicators and other sociodemographic factors were not associated with number of adults living in the household.

Among preschoolers, age was associated with anemia. For every additional month of age, the odds of anemia fell by 10%, [OR=0.90; 95%CI (0.81-0.99)]. No other variables were associated with anemia in preschoolers.

DISCUSSION

Over half (56%) of infants/toddlers and 12% of preschoolers had anemia, illustrating a severe public health concern, especially among infants/toddlers. Even though children from this study had a length or height-for-age z-score <-1 SD at recruitment, findings from this study are close to the age-related national averages reported in the latest 2014 Maternal and Child Survey in Guatemala (children ages 6-11 mo= 64.3%; 12-23 mo=39.6%; 36-47 mo=14.8%, and 48-60=9.1%) [19].

Micronutrient deficiencies were frequent in both infants/toddlers and preschoolers, with the prevalence of iron and zinc deficiency over 70% and 36%, respectively. ID in infants was comparable with that reported by the 2008-2009 micronutrient survey in Guatemala, (80.8% for <12-month-old infants with no inflammation). In contrast, the current analysis found a much greater prevalence of ID in preschoolers at 75.0% vs. <10.0% [100], either due to our selection of children with HAZ < 1- or due to an underestimation of ID in the National Micronutrient Survey as they used only ferritin and AGP1. The present study also found low rates of ferritin in preschoolers (14.3%).

The prevalence of zinc deficiency in the current analysis was similar to the rates of the Southwestern region in Guatemala reported by the Micronutrient Survey (36.8%) [100], and slightly higher than the rates of zinc deficiency from Mexico and Ecuador, (27.5%, and 28.8%, respectively). It is expected that Guatemala would have higher prevalence of zinc deficiency, as stunting, (a functional indicator of zinc deficiency) [21], is more frequent in Guatemala at ~47%, compared to 14% in Mexico, and 29% in Ecuador [113].

In contrast to the lack of association between ID and anemia, the current study found a strong association between zinc deficiency and anemia in infants/toddlers. The odds of anemia were >3 times greater for infants/toddlers with zinc deficiency. To our knowledge, few investigations have examined the relationship between zinc and anemia. In school-aged children from New Zealand, zinc deficiency increased the probability of anemia by 5 times and with no association between ID and serum ferritin [114]. A study among preschoolers in Vietnam reported a 45.7% prevalence of zinc-deficiency and anemia, with no association between anemia and iron status [115].

There are several mechanisms potentially linking zinc deficiency and anemia. Zinc is part of more than 100 enzymes and transcription factors involved in DNA, heme synthesis, and RNA translation; processes involved in growth, cell division, and erythropoiesis [52]. Zinc also is known for stabilizing erythroid cell membranes [54], and acts as a catalyst of the enzyme alpha–aminolevulinic acid dehydratase, which modulates erythroid transcriptional gene expression, supports the proliferation of immature erythroblasts, and influences development of erythroid stem cells [55]. Also, the lifespan of red blood cells is reduced in the presence of zinc deficiency due to the compromised function of zinc-dependent antioxidant enzymes [116].

Although no association was found between anemia and ID, infants/toddlers with or without anemia and preschoolers showed high rates of ID.

A recent study in India found a weak association between iron intake and anemia, suggesting that other factors might be playing a more important role in the etiology of anemia than previously thought [41].

Iron deficiency occurs when total body iron is reduced, and iron stores are exhausted [117]. When hemoglobin concentrations in erythrocytes decrease, their capacity to bind and carry oxygen is jeopardized. This impacts the peripheral tissue oxygenation homeostasis and triggers a series of physiologic responses detailed elsewhere [118]. Post-mortem and animal studies suggest that when ID is present, bioavailable iron is prioritized into erythrocytes to favor oxygen delivery over energy production for survival [119, 120]. Thus, ID anemia is thought to be an end-stage clinical manifestation of ID. These explanations may justify a high prevalence of ID without an association with anemia as seen in the current study sample.

No biomarkers of nutritional status were associated with anemia in preschoolers.

Reasons why zinc deficiency was associated with anemia in infants/toddlers and not in preschoolers may be that infants are particularly vulnerable to micronutrient deficiencies due to their rapid growth, high micronutrient requirements, lower dietary diversity, smaller gastric capacity than older children, and an increased susceptibility to infections leading to gastrointestinal disfunction and environmental enteropathy [121]. During the first 4-6 months after birth, iron stores begin to deplete and breastmilk iron and zinc are no longer able to cover the growing infant's needs [10, 11]. By 7 months, human milk provides only 0.5 mg/day of zinc, or 20% of the estimated average requirement for a 7-12 mo-old infant, highlighting the need to incorporate micronutrient-rich foods as early as 6 months [122]. This analysis identified that less than half of <12-month-old infants reported consumption of any meat, fish, poultry, or organ meats at least once during the previous 7 days compared to >95% of preschoolers. Animal source foods are essential for obtaining absorbable forms of iron, zinc, and other micronutrients [123] suggesting that infants/toddlers from this region are particularly vulnerable to deficits of some animal-source essential micronutrients such as iron and zinc. On this matter, there is an opportunity to modify local complementary feeding guidelines as they promote the introduction of beef, fish, and viscera only after 9 mo of age [124]. Other inappropriate complementary feeding practices could be contributing to more prevalent micronutrient deficiencies in infants/toddlers, such as the common habit of providing sweetened water in infants that has been associated with stunting [73].

Structural factors and policies also impact the ability of Guatemalan families to access nutrient rich foods. Iannotti and colleagues identified zinc as the micronutrient with the highest probability of inadequacy as income decreased in Guatemala [125]. Other cultural and social aspects in the Guatemalan traditional diet can influence complementary food choices in this population. Guatemalan diets are mostly plant-based, where the high-phytate traditional-maize (mean phytate content of 840 ± 42 mg/100 g) has been the principal grain of the Mayan people for more than a thousand years and continues to be a main staple [126]. Phytates form insoluble complexes with divalent minerals (e.g. iron, zinc, and calcium) inhibiting their absorption.

Finally, local efforts to address anemia through supplementation and fortification have included iron and vitamin A but not zinc.

Among the non-nutritional findings in this study, we found an inverse association of increasing number of adults in the household with anemia in infants/toddlers. In low- and middle-income countries, large family sizes have been identified as a factor associated with malnutrition [87, 88], especially when high numbers of children live in the same household [127]. In the infant/toddler group, the odds of anemia fell 31% for each additional adult living in the same household. Yet, no association between anemia and number of children living at home was observed. Further analyses showed that households with more adults had higher asset scores, and mothers of those children also received more education. Consequently, larger families could have a higher socioeconomic status and more resources for children, as well as a stronger social support network, important for the care of infants and toddlers at home. This finding is aligned with a recent cross-sectional analysis in Uganda that found that larger households had higher incomes, and children living in these households had a lower probability of anemia [128].

Uncertainty exists as to how to measure iron deficiency in populations. A strength of this study is that it included measurements of inflammation biomarkers (CRP and AGP1), and two iron status indicators: ferritin, and sTFR. These measures provide a comprehensive status of ID in this population. The present research is novel in that it is the first in the region to analyze zinc status in the context of anemia. One limitation is that the sample was selected for infants/toddlers (age 6-24 mo) and preschoolers (age 36-60 mo) with LAZ/HAZ < -1, excluding children with severe stunting or severe anemia, and therefore is not representative of the population of children under age 5 years. Finally, this study did not test for intestinal parasites (amoebas, giardias and soil-transmitted helminths) endemic to the region [129] and other secondary and prevalent factors of undernutrition.

In the context of this study, households with dirt floors, and lack of access to appropriate sources of water and sanitation are a reality. In the study location, animals (poultry, and other domestic animals) typically inhabit homes where family members eat, cook, and sleep, potentially contributing to the disease burden.

Further research understanding family structures, access to animal source foods, and nutrition supplements could clarify some remaining questions. Developing strategies to increase consumption of iron and zinc-rich foods in young children is needed. Revising local complementary feeding guidelines, educating health professionals and implementing culturally relevant education approaches that target caregivers, healthcare providers, and community leaders are some opportunities to address undernutrition in the region. Also, if food sources of iron and zinc are unavailable or inaccessible, consideration of incorporating iron/zinc supplements during the complementary feeding period would be an inexpensive alternative that could improve the nutritional status of young children in rural Guatemala. Finally, in locations with a high prevalence of stunting and phytate-rich diets, assessment of zinc should be considered as part of the battery of tests to identify underlying causes of anemia.

Chapter 3: Higher Prevalence of Anemia in Urban Compared to Rural Children of Haiti Mediated by Helminth Infection Morbidities

ABSTRACT

Background: In Haiti, urban school-aged children have a higher prevalence of anemia compared to rural children; 71% vs. 51%, respectively.

Objective: To compare characteristics between urban and rural Haitian school-aged children and identify mediating factors between urban and rural residence and anemia.

Methods: This cross-sectional study used baseline data from two cluster-randomized controlled trials assessing the effectiveness of a nutrition intervention in school-aged children attending schools in Cap-Haitien and surrounding rural areas. The study was registered at ClinicalTrials.gov as NCT02747524. A total of 300 rural children and 981 urban children were included in this analysis. Generalized linear mixed models were conducted to identify significant associations with anemia (the dependent variable), adjusting for age, income and school cluster. A final step consisted of including significant variables in a mediation analysis to explore relationships between rural/urban residence and child anemia.

Results: Models showed that in rural children, stunting was positively associated with anemia [OR=3.42; 95%CI (1.47-7.97)], while households with more adults lowered the odds of anemia [OR=0.74; 95%CI (0.62-0.89)]. Among urban children, intestinal worm morbidities increased the odds of anemia [OR=1.74; 95%CI (1.13-2.68)]. In both urban and rural sites, older age decreased the odds of anemia, [OR=0.88; 95%CI (0.81-0.96)] and [OR=0.93; 95%CI (0.88-1.00)], respectively. Helminth infection morbidities partially mediated the relationship between anemia and rural-urban residence (b=0.10, SE=0.05, P=0.037).

Conclusions: Anemia is a severe public health problem in school-aged children from Haiti. This study identified key differences between urban and rural children that should be considered when developing targeted and cost-effective interventions to improve local nutritional and health status of this population. Particular attention should be paid to deworming measures in children residing in urban areas.

INTRODUCTION

Haiti is the poorest country of the Americas and the Caribbean, and one of the countries with the highest income inequality in the world (Gini of 60.8) [13]. More than half of the population lives under the poverty line, and the economy is heavily dependent on foreign aid. In terms of health, Haiti has one of the highest prevalence rates of anemia, 66.3% for children under the age of 5 years and 49.0% for women ages 15 to 49 years [26, 130]. In addition, 21.9% of children exhibit stunting (i.e. height-for-age z score <2) and 9.5% have wasting (i.e. weight-for-height <-2).

Young children and women are particularly vulnerable to nutritional deficiencies. The largely irreversible and negative long-lasting impacts of undernutrition and anemia during gestation and throughout the first years of life [96] provides compelling rationale for the focus on these specific subgroups. However, school-aged children living in poverty are still vulnerable, and nutritional deficiencies during childhood have been associated with poor academic and cognitive performance, lower work capacity, impaired learning and cognitive development [7, 131-133].

A considerable body of literature worldwide reports that children residing in urban locations tend to have better nutritional and health status, as compared to those living in rural areas [29, 83]. Yet, a few studies from Haiti have documented higher proportions of anemia among urban populations of children and women compared to those living in rural areas [30-32]. In previous analyses, we found that among children from urban, resource-poor communities, vitamin A supplementation and deworming were positively associated with increased hemoglobin concentrations [31]. Fever showed a negative association with hemoglobin, and stunting increased the odds of anemia by 1.48 in this population of Haitian children from urban residence [31]. At present, anemia in rural children from Haiti remains poorly characterized.

The discrepancy of anemia between urban and rural locations has been previously documented [30]. A study by Heidkamp et al. [30] observed a significantly larger proportion of anemia in Haitian young children from urban vs. rural areas; 65% vs. 56%, respectively [30]. To our knowledge, the differences in the prevalence of anemia in rural vs. urban children from Haiti have not been examined.

The objectives of this manuscript are to characterize anemia in rural Haitian children, and to identify possible mediating factors that may influence the urban-rural differences in the prevalence of anemia.

METHODS

A secondary cross-sectional analysis of data was performed. Original trials tested the efficacy of a fortified peanut butter-based food product on child growth and anemia status in a population of school-aged children from Cap-Haitien and rural areas (~120 km south) [93, 134]. Both trials were approved by the National Bioethics Committee of the Ministry of Health in Haiti and the Institutional Review Board of the Human Research Protection Office of Washington University in St. Louis. The present research was approved by the University of Texas at Austin Institutional Review Board FWA # 00002030. Findings from the original trials have been published [32, 135] and the ClinicalTrials.gov registration number is NCT02747524.

Design of Study and Participants

The urban study was conducted in Cap-Haitien, with an estimated population of 500,000 inhabitants. The city is located in the Nord department by the Mapou River [136]. For this analysis, only baseline data, collected in December 2012, was utilized. A total of six schools were selected, and participants were included based on the following criteria: ages 2.5 to ≤13 years; no fever, congenital health condition, or peanut or soy allergy; not suffering from severe wasting (i.e. weight-for-height z-score <−3); registered at school in 2012–13, and having completed the baseline survey.

The rural study was conducted in three schools from two rural communities, approximately 120 km from Cap-Haitien but still in the Nord department. Based on formative research, the communities and schools were considered representative of the Haitian rural livelihood for external validity [32]. Data was collected in December 2014. Similar to the urban trial, only the baseline data was included in these analyses. Children were ages 2.5 –16 years (n=321); this age span was broader because there were fewer children and less population density. Children from rural areas were then selected based on the same eligibility criteria, and school registration for the year 2014–15.

For the present analysis, children from both trials between 2.5 to ≤13 years old were included, in order to have the same age ranges from both samples. A total of 1,281 children, 300 of rural and 981 of urban residence, were identified to meet inclusion criteria.

Measurements

A survey that included socioeconomic, demographic, water, sanitation, hygiene, child diet, and morbidity concerns was administered. Morbidity was determined with a 15-day recall for diarrhea (≥3 semi-solid or liquid stools in 24 hours) and fever. Monthly recalls were used for intestinal worm morbidities, cough, and ear infections; and 3 month recalls for malaria. Dietary information was collected through a 24-hour food frequency of intake using common foods identified in the formative research stage [20].

Drawing from formative research conducted in the sites, food lists were developed, and foods grouped into 17 categories. These included cassava bread, white wheat bread; cereals, (rice, maize, wheat, millet, sorghum, spaghetti, and other pastas); roots and tubers (cassava, potatoes, yams, cooking bananas, other); beans and other legumes (beans, peas, groundnuts, grams, other); eggs; milk (cow, goat); yogurt; cheese and other dairy products; poultry (chicken, duck, guinea fowl, other); meat (beef, goat, pork, lamb, other); fish and shellfish (large and small fish); fruits; vegetables; oils, butter, and other fats; crackers; and cookies. Dietary diversity was calculated by adding the food types consumed in the previous 24 hours [137]. The score ranged from 0 to 17, and higher numbers represented more diverse diets. The "fresh fruits and vegetables" variable was determined by adding consumption of the "fruits" and "vegetable" food groups. The "animal source foods" variable was created by adding "meat, fish and shellfish, yogurt, cheese and other dairy products, eggs and poultry." The variable "owns livestock" consisted of affirmative answers to questions concerning owning poultry, cows, horses, mules, and goats/pigs. Income and wealth questions included reception of money transfers from abroad, income, and an asset questionnaire [135].

Hemoglobin was collected from capillary blood by trained staff and analyzed in the field, using a previously calibrated portable spectrophotometer (Hemocue 201a, Brea, CA), following the manufacturer's instructions [138]. Standing height was measured with a stadiometer (ShorrBoard, Weigh and Measure, LLC, Olney, MD) to the nearest 1 mm. Weights were measured to the nearest 0.1 kg, with a digital weight scale (Seca Model 874, Chico, CA) following standard protocols [139].

Anthropometric z-scores were calculated based on the 2006 World Health Organization's Child Growth Standards for children <5 years of age, and Child Growth References for older children [9, 140].

Analysis of Statistics

Data collected was entered in a database and imported to SPSS® statistical software package version 24.0 (IBM SPSS Inc., Chicago, IL, USA) for this analysis.

A literature review was performed to identify sociodemographic, dietary and maternal/child variables that showed a relationship with anemia and place of residence (dependent variables) through binary logistic regressions (Table 3). Variables available from the survey and that were previously identified in the literature review were included in a univariate analysis using binary logistic regressions. Factors included in this first step are listed in Table 3.

Table 3: Sociodemographic and morbidity characteristics of rural and urban Haitian school-aged children[1,2]

	Rural				**Urban**				**Urban vs. Rural**
	Anemia				**Anemia**				
	No n=143	**Yes n=157**	**All n=300**	***P***	**No n=241**	**Yes n=740**	***All n=981***	***P***	***P***
Child Characteristics									
Anemia, %	47.7	52.3			24.6	75.4			**<0.001**
Severe anemia, %	99.0	1.00			97.0	3.0			0.070
Hb, g/dL	12.28±0.77	10.15±0.93	11.17±1.36	**<0.001**	12.16±0.64	10.05±1.15	10.57±1.39	**<0.010**	**<0.001**
Age, yr	8.01±2.71	7.22±2.89	7.60±2.83	**0.017**	8.35± 2.37	8.20±2.50	8.23±2.46	0.411	**<0.001**
Female sex, %	45.1	50.3	47.8	0.365	51.9	51.6	51.7	0.947	0.243
Sociodemographic									
Maternal education (≥ secondary), %	33.8	32.9	33.3	0.870	36.9	36.4	36.5	0.899	0.324
Income (>501 Gourdes)[3], %	46.1	39.6	42.7	0.261	34.2	33.6	33.8	0.883	**0.005**
Remittances	14.8	20.0	17.5	0.239	21.7	19.9	20.3	0.579	0.288
Children living in household, N	4.04±1.70	3.92±1.64	3.98±1.65	0.535	3.29±1.54	3.36±1.64	3.34±1.61	0.604	**<0.001**
Adults living in household, N	3.30±1.54	2.83±1.29	3.05±1.43	**0.005**	2.90±1.59	3.10±2.06	3.05±=2.00	0.204	0.996

Table 3, cont.

Poultry ownership, %	53.5	47.8	50.5	0.321	13.8	12.8	13.1	0.703	**<0.001**
Livestock ownership, %	75.4	72.0	73.6	0.509	19.8	17.3	18.0	0.411	**<0.001**
Water and Sanitation									
Tap water at home, %	3.5	3.2	3.3	0.872	25.8	25.6	25.7	0.945	**<0.001**
Automatic Toilet/Flush	0.0	0.0	0.0	-	7.9	6.1	6.5	0.329	0.997
Open defecation or other	32.9	33.8	33.3	0.870	18.3	18.2	18.2	0.996	**<0.001**
Treats drinking water, %	82.0	83.4	82.8	0.755	69.6	69.3	69.4	0.942	**<0.001**
Total people using same latrine	8.30±2.60	8.30±3.38	8.30±3.04	0.995	8.77±4.12	9.40±4.50	9.24±4.41	0.080	**<0.001**
Nutritional and Health Status									
Stunted, %	6.3	16.6	11.7	**0.008**	11.1	15.0	14.0	0.137	0.310
Underweight, %	6.8	15.1	11.4	0.055	11.3	15.5	14.4	0.172	0.237
Fever (past 2 weeks), %	6.3	4.5	5.4	0.481	19.2	25.1	23.6	0.067	**<0.001**
Malaria (past 3 months), %	0.0	0.6	0.3	1.00	7.5	10.1	9.5	0.219	**<0.001**
Diarrhea (past 2 weeks), %	3.5	2.5	3.0	0.624	15.4	11.0	12.0	0.070	**<0.001**
Intestinal worm morbidities (past 1 month), %	1.4	0.7	1.0	0.524	13.9	21.9	19.9	**0.011**	**<0.001**

Table 3, cont.

Deworming treatment (past 6 months), %	65.0	64.7	64.9	0.963	74.5	78.0	77.2	0.286	**<0.001**
Dietary									
Dietary diversity score[4]	4.42± 1.59	4.37±1.71	4.39±1.65	0.790	4.67±2.12	4.65 ±2.28	4.66±2.24	0.937	0.063
Any animal source food, %	27.9	23.9	25.8	0.429	37.5	42.1	41.0	0.235	**<0.001**
Beef, fish and shellfish, poultry and organ meats, %	16.2	9.7	12.7	0.100	22.7	25.2	24.5	0.465	**<0.001**
Cereals, %	90.8	89.7	90.2	0.750	86.1	84.8	85.1	0.641	**0.029**
Fresh fruits and vegetables, %	51.8	57.1	54.6	0.366	52.8	51.1	51.5	0.673	0.365
Vitamins (past 6 months), %	13.8	20.7	17.2	0.220	28.6	25.2	26.0	0.293	**0.011**

[1] Values are means (SDs) unless otherwise indicated.
[2] Groups significantly different by t- or univariate binary logistic regressions.
[3] 501 Haitian Gourdes was equivalent to ~10 USD at the time of data collection (December 2012 and 2014).
[4] Swindale, Anne, and Paula Bilinsky. 2006. Household Dietary Diversity Score (HDDS) for Measurement of Household Food Access: Indicator Guide (v.2). Washington, D.C.: FHI 360/FANTA.

Next, independent variables with plausible connection to anemia identified in the previous step were tested using generalized linear mixed models (GLMM) with binary outcomes (Table 4). Each model was controlled for school cluster, age, and income.

Table 4: Significant variables for model of anemia, each adjusted by cluster, age and income.

Variable	All children[1]		Rural		Urban	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Urban residence	2.35 (1.10-5.01)	0.027	-	-	-	-
Age	0.92 (0.87-0.96)	<0.001	0.88 (0.81-0.96)	0.004	0.93 (0.88-1.00)	0.046
Intestinal worm morbidities	1.66 (1.09-2.54)	0.019	0.49 (0.04-6.30)	0.584	1.74 (1.13-2.68)	0.013
Treats drinking water	0.95 (0.71-1.29)	0.756	0.89 (0.46-1.72)	0.726	0.97 (0.69-1.36)	0.85
Adults living in household	1.00 (0.93-1.07)	0.889	0.74 (0.62-0.89)	0.001	1.06 (0.97-1.16)	0.202
Malaria[2]	1.43 (0.83-2.46)	0.198	-	-	1.42 (0.83-2.45)	0.205
Stunting	1.84 (1.22-2.79)	0.004	3.42 (1.47-7.97)	0.005	1.49 (0.93-2.37)	0.097

[1] Models also adjusted by rural or urban residence
[2] One case of malaria reported in rural area

The final step constructed a mediation analysis model (MPlus Version 7.4, Los Angeles, CA) [141]. Independent variables that remained significant (alpha <0.05) from the GLMM in the previous step were included in the mediation model to assess the relationships between place of residence and anemia. All mediators and dependent variables were adjusted by age and income in the model.

Sensitivity analyses were performed to confirm the significant associations with the variables through unadjusted univariate GLMM with anemia (dependent variable) to the following variables: income, stunting, underweight, intestinal worm morbidities and number of adults living in the same household.

RESULTS

Characteristics of children from urban and rural residence are listed in Table 1. Anemia was present in more than one-half of Haitian children from both rural and urban areas. The prevalence was significantly higher in urban children compared to rural, 52% and 75%, respectively. Children were distributed evenly by sex; however, rural children were ~6 months younger than those living in urban areas.

More than one fifth of rural homes had a combined monthly income of ≥501 Gourdes (equivalent to ~10 USD) compared to urban households, P=0.005. Greater income was significantly associated with livestock ownership [OR 1.37; 95%CI (1.01-1.84), P=0.041], even after adjusting by residence and school cluster. A significantly higher number of children were living in rural households, as compared to urban households.

No differences were observed in maternal education and remittances between rural and urban areas. In rural locations, the number of adults living in the household was significantly lower in the homes of children with anemia, as compared to those without anemia. The ratio of people per room was significantly greater in urban vs. rural areas (median of 3.0 for urban and 2.0 for rural, P<0.001). The number of rooms was lower in urban households by a magnitude of one. As expected, about one in two rural households owned poultry, as compared to one in seven urban households, P<0.001. For this variable, no differences were observed between anemia status in either context.

Urban homes had eight times greater access to tap water than did rural households. However, more rural participants reported always treating drinking water through chlorine/aquatabs or boiling water, as compared to urban: 82.8% vs. 69.3%, P<0.001, respectively. The number of people using the same latrine was significantly higher in urban vs. rural households, and open defecation practices were more frequent in rural homes, P<0.001. Considering that tap water at home could be perceived as "safe to drink without additional water treatment," further exploration between water treatment and tap water was performed. About 70.2% of households with no tap water reported treating the water vs. 67.1% of households with tap water. The association was not significant, P=0.400.

The prevalence of stunting or underweight between children from urban or rural areas did not differ. However, stunting increased the odds of anemia by 3.4 times in rural children.

Fever, diarrhea and malaria were more frequent in urban children; but any of the symptoms were associated with anemia status for either place of residence. Intestinal worm morbidities were more prevalent in urban children with anemia vs. no anemia; and were also significantly more frequent in urban vs. rural children, 20.5% vs. 1.0%, P<0.001 respectively.

Only one rural child with anemia was reported to have had malaria in the past 3 months. A larger prevalence was found in children residing in urban areas, with 7.5% of children with no anemia, and 10.1% of children with anemia. But no differences were found between anemia status in urban areas. A significantly larger proportion of urban children received deworming treatment in the past 6 months, as compared with those from rural areas; but no differences were observed between anemia status and deworming.

Dietary diversity scores were similar in urban and rural children and did not differ by anemia status. Almost twice as many urban children reported eating foods from animal sources, compared to those from rural areas.

Vitamin supplementation in the previous 6 months was more frequent in urban children, compared to rural children, 26.0% vs. 17.2%, P=0.011, respectively.

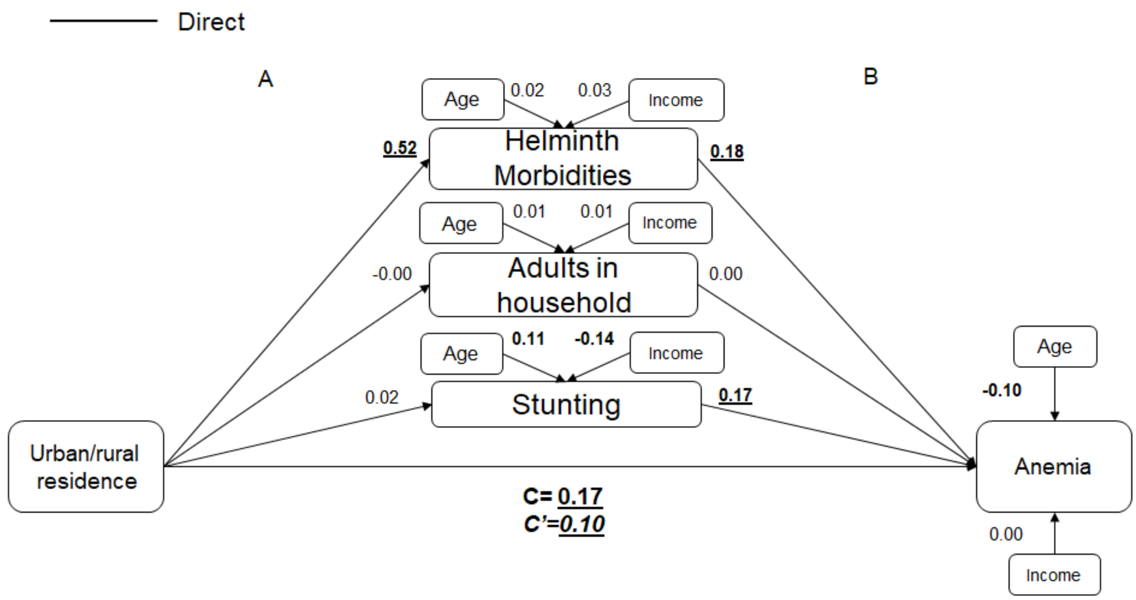
Factors Associated with Anemia

Table 4 depicts the factors associated with anemia in both urban and rural children. Overall, living in an urban setting increased the odds of anemia by 2.35, and more age reduced the odds of anemia by 8%. Helminth infection morbidities were positively associated with anemia, only in children living in urban locations. In rural children, older age lowered the odds of anemia by 12%, and increasing numbers of adults reduced the odds of anemia by 26%. Conversely, rural children with stunting had 3.4 times greater odds of anemia.

Factors Mediating Place of Residence and Anemia

A mediation model (Figure 3) was used to investigate the hypothesis that helminth infection morbidities, number of adults living in the same household and stunting were mediating the effect of "place of residence" (urban vs. rural) on anemia. Standardized results indicated a significant and positive association between urban residence and intestinal worm morbidities (b= 0.52, SE= 0.05, P<0.001) (path A). Stunting (b=0.17, SE=0.06, P=0.007), and age (b=-0.10, SE=0.04, P=0.008) were positively associated with anemia (path B). A significant indirect effect between urban residence and anemia also was observed (b=0.10, SE=0.05, P=0.037) (path C'). The direct effect between urban residence and anemia remained significant (b=0.17, SE= 0.06, P= 0.004).

Figure 3. Mediation analysis showing standardized coefficients of variables included in the model [1-3]



[1] χ^2=2.33, df=3, p=0.51; CFI = 1.00, TLI=1.03; RMSEA =0.00, 90%CI (0.00-0.04). Pathway A: predicts child variables (helminth morbidities, number of adults living in the household, and stunting) by urban/rural residence; Pathway B: predicts anemia (the dependent variable) by child mediating variables; Pathway C'(indirect): tests whether effects of residence on anemia are mediated by helminth morbidities, number of adults living in household and stunting.
[2] Direct standardized estimates.
[3] Estimates in bold and underlined, p<0.05

Sensitivity analyses with non-adjusted generalized linear mixed models were performed to confirm findings. Unadjusted models were similar for stunting in rural children, OR=1.77; 95% CI (1.18-2.65), and helminth infection morbidities for urban children, OR=2.18; 95% CI (1.47-3.22). The variable "number of adults living in the same household" was no longer significant in the unadjusted model, P=0.860. The mediation model was run, excluding "number of adults in the household." This analysis yielded similar results to the adjusted mediation analysis shown in figure 1: χ2=0.27, df=1, p=0.61; CFI = 1.00, TLI=1.01; RMSEA =0.00, 95% CI (0.00-0.06). Urban residence was still significantly associated with helminth infection morbidities (b= 0.56, SE= 0.06, P<0.001) (path A). Stunting (b=0.18, SE=0.06, P=0.004), helminth infection morbidities (b=0.19, SE=0.08, P=0.015) and age (b=-0.107, SE=0.04, P=0.008) were significantly associated with anemia (path B). A significant total indirect effect between urban residence and anemia was observed b=0.25, SE=0.12, P=0.032 (path C'). The direct effect between urban location and anemia also was significant (b=0.14, SE= 0.06, P= 0.021).

Discussion

These results suggest that the odds of anemia were 2.35 times greater for children living in urban areas, as compared to those residing in the countryside. Stunting increased the odds of anemia in children from rural areas by 3.4 times and had a significantly lower infectious burden than children living in Cap-Haitien. Intestinal worm morbidities partially mediated the relationship between place of residence and anemia and increased the odds of anemia in urban children.

The proportion of anemia for urban children (75.4%) from this sample appears to be the highest reported for any Latin American and the Caribbean country or program in recent times [27]. Similar findings were described by Heidkamp et al. 2013 using data from the Demographic Health Survey in 2005/6 [30].

To explain the urban-rural gap in anemia, this study hypothesized that poor sanitation and hygiene could result in increased infectious morbidity and anemia in urban vs. rural locations. In Haiti, urban areas are highly populated and lack appropriate sanitation and access to potable water. Even though most sanitation and hygiene variables observed in this study were lower for rural households, morbidity was significantly greater in urban children. For instance, the urban sample reported more intestinal worm morbidities, as compared with rural children. This variable was also associated with a greater odds of anemia in urban children only.

Among all the variables analyzed in this study, the mediation model reported that helminth infection morbidities partially mediated the relationship between place of residence and anemia. This finding relates to a longitudinal analysis by Iannotti et al. [31], where deworming was associated with increasing hemoglobin concentrations in a sample of 1,047 children from Cap-Haitien [142]. Other studies in similar contexts and different populations have found a significant association between intestinal worm infections and anemia [143-145].

Intestinal parasites are a primary cause of diarrhea, dysentery (blood associated with diarrhea) and intestinal malabsorption in the developing world [63]. Co-infection with multiple parasites is significantly more frequent than single infections. In children from the Nord department of Haiti, Champetier de Ribes reported a prevalence of helminth infections of 45.2%, [60], with a 28% prevalence of co-infection with two or more helminths [60]. Other studies in Haiti have observed that 90% of children with helminth infections had a protozoan co-infection [62]. These intestinal multi-parasitic infections elevate the probability of anemia and diminish hemoglobin concentrations, even more so than single infections [66]. Polyparasitism may be common in this population, and urban children may be more susceptible due to the population density in urban areas [146]. Intestinal parasites may be directly causing anemia through gastrointestinal blood loss, and through the promotion of intestinal inflammation and environmental enteric dysfunction [147]. As a consequence of chronic inflammation, nutrient absorption may be reduced, with subsequent systemic inflammation. Chronic systemic inflammation promotes anemia via hepcidin activation, an acute phase protein that inhibits duodenal absorption of iron and reduces its release into the circulation [57, 148].

This investigation also observed that children residing in urban areas reported fever, malaria, and diarrhea much more frequently than those from rural areas. These results are similar to the latest Haiti survey that documented slightly higher morbidity rates in urban vs. rural children under 5 years: fever, 32.8% vs. 30.8%; and diarrhea, 22.6% vs. 20.5% [26].

Another hypothesis proposed by Heidkamp et. al. [30] was that people from rural areas from Haiti would have greater access to seasonal micronutrient-rich fruits and vegetables at low- or no-cost, vs. those from urban locations. In contrast, urban populations may have had more access to energy-dense cereal staples [30]. Yet, the present research observed the opposite, with rural children having greater consumption of cereals. No differences in the intake of fresh fruits or vegetables were observed, based on area of residence (Table 1). This analysis also explored whether children residing in rural areas would have a greater intake of animal source foods. Animal source foods are rich sources of essential and bioavailable micronutrients that are difficult or impossible to obtain via plant sources. Potential problem nutrients might be vitamin A, vitamin B12, riboflavin, iron or zinc [123, 149]. In rural areas, the rearing of animals is an ubiquitous practice [33], and studies have linked livestock ownership with increased intake of meat and dairy [34] and better nutritional status in children [150]. As expected, a larger proportion of rural households owned livestock; however, intake of meat, dairy and eggs was more frequent in children from urban residence. Findings from this study are similar to a study in Ghana, where livestock ownership was not associated with increased intake of animal source foods [151].

In addition, livestock ownership has been associated with increased household incomes [34], as was observed in this research. Engle [152] suggested that increased income by itself may lower anemia either by improving dietary quality and/or by possible increasing expenditures in other wellbeing activities [152]. It is plausible that resources in rural households may be directed towards improvements in water treatment, health and disease prevention, vector management, or other aspects that could ameliorate the anemia burden in rural children.

In this investigation, stunting increased the odds of developing anemia by 3.4 times in rural areas. Using a similar sample of urban children from Cap-Haitien, a longitudinal analysis by Iannotti et. al., [31] also observed an association between stunting and anemia, suggesting that in Haiti, chronic micronutrient deficiencies may be important in both urban and rural settings.

The co-occurrence of stunting and anemia is common in low- and middle-income countries [153-155]. However, a recent multi-country analysis observed that, although both anemia and stunting may share some basic factors, both conditions may be more independent than commonly assumed. This suggests that interventions developed to address either condition, in isolation of the other, may fail to improve the other [156].

Previous studies in Uganda [128] and Guatemala [157] reported that increasing numbers of adults in the household were inversely associated with anemia. The present analysis observed that rural households with more adults lowered the child's odds of anemia by 26%. Living with additional adults may result in additional total income and resources that could be spent for the children [157].

A limitation of this study is that the urban and rural trials occurred in different years. Yet there does not appear to be any major environmental, socio-economic, or other significant events occurring in the two years that may have driven differences in anemia. Dietary and infectious information were reported by the primary caregivers, which could result in reporting biases. Also, intestinal worm morbidities were not confirmed by fecal sample analysis, such that intensity of infections could not be determined. Both could be potential sources of reporting bias, as well as an underestimation of the intestinal parasite morbidity burden. Also, this research did not collect nutritional or inflammatory biomarkers associated with anemia which could have provided insight into anemia etiologies as well. Finally, the present research did not contemplate inherited hemoglobin disorders that may be important contributors of anemia in this population. A study examining the genetic structure of populations from Haiti observed that Haitians exhibited minimal contributions from non-African populations in their gene pool. This suggests that Haitians may reflect similar prevalences of hemoglobinopathies as do populations from Sub-Saharan Africa, which are highly prevalent [23]. Finally, a report from the Ouest department in Haiti observed a prevalence of ~20% of glucose 6 phosphate dehydrogenase deficiency in a population of school-aged children further suggesting that hemoglobinopathies may be prevalent in this population [71].

In conclusion, this research suggests that non-nutritional factors might be central to the etiology of anemia in school-aged children from urban areas of Haiti. It is plausible that intestinal worm morbidities may be a primary factor of anemia in these children, rather than just diet. To our knowledge, this is the first study that aims to explain the existing urban-rural differences in anemia rates among school-aged children from Haiti. This information can facilitate the development and implementation of cost-effective programs targeted to improve nutritional and health status of Haitian children. Specifically, systematic deworming and antiprotozoal medications every six months should be considered and coupled with interventions focused on improving the wellbeing of children.

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Chapter 4: Conclusions

The research results obtained during the course of this dissertation suggest that both Guatemala and Haiti face significant anemia burdens that require study in order to design context- and population-specific interventions. To our knowledge, this is the first study to use biomarkers to better understand the prevalence rates of anemia among rural children in Guatemala, and the first time a mediation analysis was employed to explain the differences in anemia rates between urban and rural children in Haiti.

The study in Guatemala highlights that other micronutrients aside from iron, as well as other non-nutritional variables, may have highly significant roles in the development of anemia in young children. Iron deficiency was present in more than 75% of children under 5 years, and zinc deficiency was present in more than 60% of infants/preschoolers and toddlers. Therefore, nutrition supplementation involving multiple micronutrients may provide significant benefit to this population, more so than iron/vitamin A alone or a bi-annual micronutrient powder supplementation prophylaxis.

In Haiti, children living in urban areas had 2.4 times increased odds of developing anemia compared to rural children, and intestinal helminth morbidities increased the odds of anemia in children living in urban locations. Although not associated with anemia, children from urban areas had a significantly higher prevalence of other morbidity symptoms, including diarrhea and fever, compared to rural children. On the contrary, stunting was positively associated with anemia only in rural children. Overall, the Haitian study found that other non-nutritional factors may be playing a more important role in the etiology of anemia in school-aged children, especially in urban areas. More research is needed to identify appropriate and cost-effective interventions to improve anemia and nutritional status in children from rural and urban areas of Haiti. In the meantime, comprehensive programs that seek to reduce gastrointestinal morbidity should be prioritized, in addition to any nutrition supplement for this population.

In rural areas of both countries, family structure may have a meaningful influence on children anemia status. The number of adults living in the household reduced the odds of anemia in both locations. This finding suggests that other social factors and caregiver practices may play a significant role in anemia status in rural populations. Further research efforts are needed to the determine the household mechanisms for reducing anemia, including the amount of resources brought to the household, the family care opportunities, quality and time spent in meal preparation and child feeding, and the time and resources spent in healthcare and education of children. A better understanding of household dynamics and childcare practices could influence targeted interventions.

This analysis also uncovered other areas requiring further study. Among all the factors analyzed preschoolers from rural Guatemala, only lower age increased the odds of anemia. Lack of association among biomarkers in this age category suggests that other variables not analyzed in this study may be more important for this age group. Elevated inflammation biomarkers in preschoolers were observed more frequently than in infants and toddlers, suggesting that anemia in preschoolers may be more closely related not only to nutritional deficiencies but also to morbidity and chronic inflammation. Future studies should consider including more detailed morbidity information to address the data gap that this present study could not resolve, including the characterization of the gastrointestinal morbidity, and biomarkers of environmental enteric disease.

In the case of Haiti, several questions remain unanswered from this study. First, the role of hemoglobinopathies and anemia in urban and rural populations of Haiti is unknown; however it is thought to be similar to the prevalence rates of Central and East Africa, given the Haitian population origins [23] and other studies that have been performed in the region

[71]. Further, the role of nutrition biomarkers and inflammatory status in the etiology of anemia, and the evaluation of local policies addressed to reduce burden and improve sanitation in both urban and rural contexts are important areas to investigate.

Anemia remains a significant health issue in the Americas, and interventions to address the disease that primarily affects the poor and underserved should be given a priority, especially given the negative short- and long-lasting impacts anemia and undernutrition have on individual health and social development. Anemia and undernutrition during childhood have been associated with poorer academic performance that results in decreased work, learning, and earning capacity of individuals later in life, when compared to individuals who did not suffer nutritional disorders during childhood [96-98]. When more than the 50% of the population is not reaching their full developmental potential due to preventable and/or treatable conditions, the socioeconomic implications in the development of entire regions can be profound, perpetuating the cycle of poverty and increasing inequality in countries like Haiti and Guatemala [158].

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