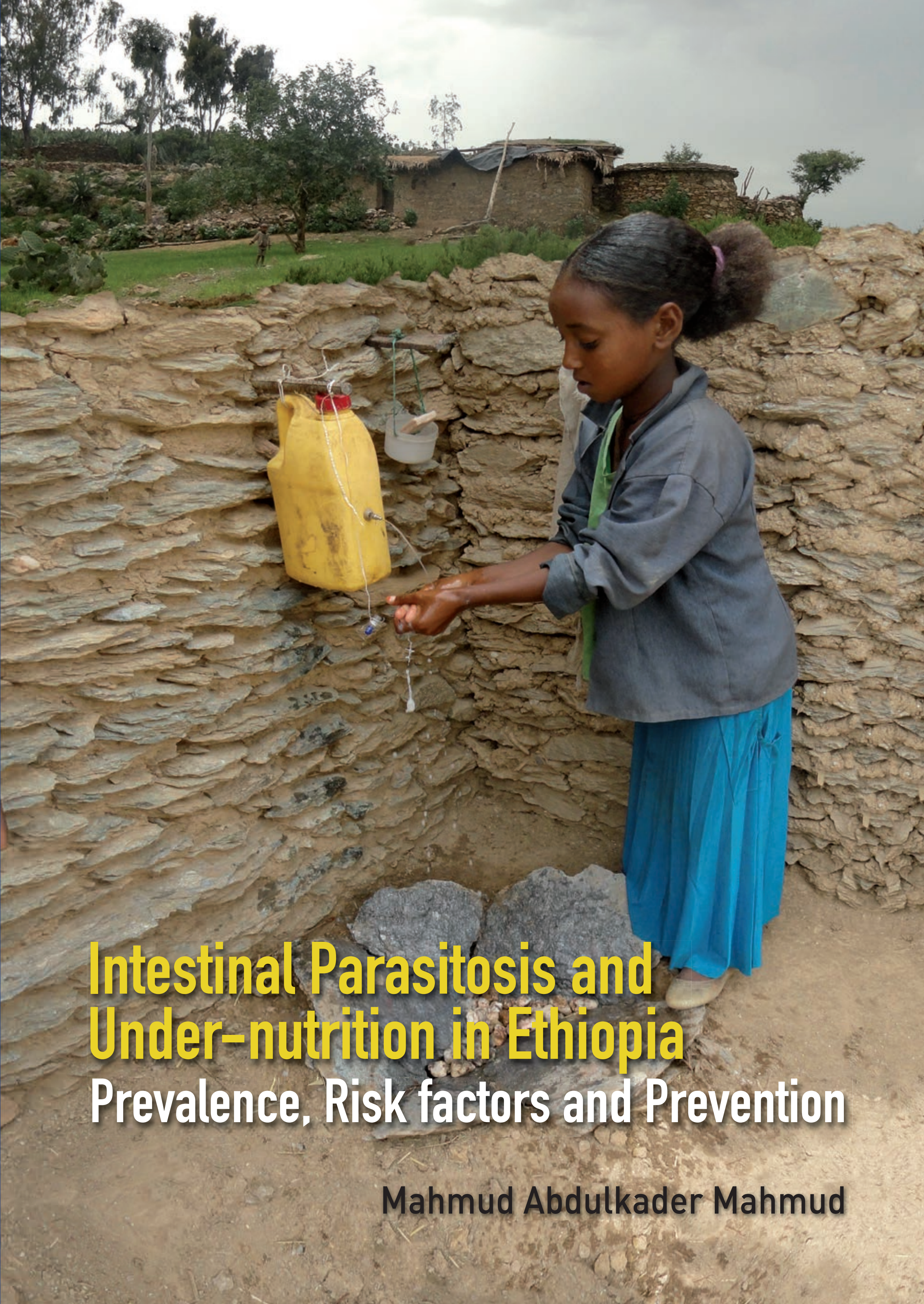
Intestinal Parasitosis and Under-nutrition in Ethiopia: Prevalence, Risk factors, and Prevention



Intestinal Parasitosis and Under-nutrition in Ethiopia

Prevalence, Risk factors and Prevention

Mahmud Abdulkader Mahmud

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Intestinal Parasitosis and Under-nutrition in Ethiopia:

Prevalence, Risk factors and Prevention

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Abbreviations

AIDS	Acquired Immune Deficiency Syndrome
AOR	Adjusted Odds Ratio
ART	Anti Retroviral Treatment
BMI	Body Mass Index
CI	Confidence Interval
COR	Crude Odds Ratio
CSA	Central Statistics Agency
DHS	Demographic Health Surveillance
DALYs	Disability Adjusted Life Years
EPG	Eggs per gram
FMOH	Federal Ministry of Health
HDA	Health Development Army
HDSS	Health and Development Surveillance Site
HEP	Health Extension Program
HEWs	Health Extension Workers
HIV	Human Immunodeficiency Virus
IDA	Iron Deficiency Anaemia
IPIs	Intestinal Parasitic Infections
MDG	Millennium Development Goal
NaCl	Sodium Chloride
NTD	Neglected Tropical Disease
OR	Odds Ratio
PEU	Protein Energy Under-nutrition
RBC	Red Blood Cells
RCT	Randomised Controlled Trial
SD	Standard Deviation
STH	Soil Transmitted Helminth
SPSS	Statistical Package for Social Sciences
WHO	World Health Organisation

Chapter 1

General introduction

1. General introduction

Intestinal parasitic infections (IPIs)[1] and under-nutrition (both protein-energy malnutrition and micronutrient deficiencies)[2] have major health and socio-economic repercussions among populations of low socioeconomic development. Vulnerable groups such as school-aged children and immunocompromised patients are among those who suffer most from IPIs. Research on IPIs affecting children and those with Acquired Immune deficiency Syndrome (AIDS) is of major importance, as IPIs cause high morbidity and mortality rates among them, and information regarding these infections facilitates the development of better control strategies.[3] Identifying factors that increase vulnerability to infections and under-nutrition, in addition to empirical assessment of public health interventions is essential to ensure that prevention and control programmes in developing countries - where national health budgets are often chronically insufficient achieve optimal impacts.

This thesis has three main purposes. First, to determine the prevalence and associated risk factors for IPIs and under-nutrition among the most vulnerable population groups in impoverished areas of Ethiopia. Second, to assess the impact of simple public health interventions (hand-washing and nail clipping) in the prevention of intestinal parasite re-infection rates, reduction of worm intensity and anaemia among school-aged children. Third, to describe associations between parasitic infection, anaemia and diarrhoea in children, and to explore whether interventions could be effective in preventing IPIs in children of different demographic backgrounds. Current epidemiological knowledge on IPIs and under-nutrition are presented in this chapter. Based on these empirical finding and theoretical concepts, we will introduce our research aims and conclude with the outline of this thesis.

1.1 Intestinal parasitic infections and under-nutrition

IPIs and under-nutrition have distinctly similar geographic distributions, making the same people in poor communities suffer their concurrent effects.[4] In such settings, illnesses due to infectious agents and under-nutrition create a vicious cycle (Figure 1.1). Inadequate nutrient intake and nutrient losses resulting from infections negatively affect nutritional status, and poor nutritional status predisposes people to infections by lowering immune protection.[2,5,6]

The most important intestinal parasites linked to nutritional status are the human soil-transmitted helminthes (STHs): the hookworms: *Necator americanus* and *Ancylostoma duodenale*; roundworms: *Ascaris lumbricoides* and *Trichuris trichiura*; and protozoan: *Giardia lamblia* and *Entamoeba histolytica.*[1] In endemic settings these parasites contribute to poor growth, anaemia and other micro-nutrient deficiencies in children.

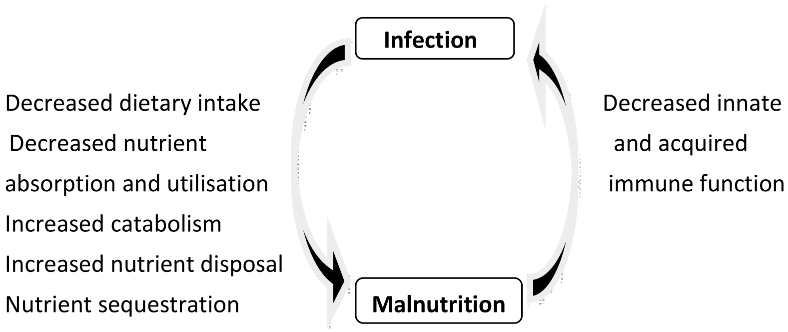

Figure 1.1 The “Vicious Cycle” of infection and under-nutrition (adapted from: Brown, 2003).

Worm infestations exert their effects through different mechanisms. Infection with human hookworms cause iron deficiency anaemia (IDA) and IDA is the most widespread nutritional deficiency worldwide.[7] Adult worms of both hookworm species attach to the mucosa of the upper small intestine and feed on blood and tissue fluids, releasing anticoagulants to maintain blood flow which can result in the development of IDA.[8] IDA is associated with decreased physical and mental development[9] and impaired immune function in children.[10] Infection with the largest roundworm, *A. lumbricoides*, results in impaired fat digestion, decreased vitamin absorption, and temporary lactose intolerance in children.[11] These mechanisms are potentially responsible for reduced growth and other developmental defects in infected children. Furthermore, co-infections with *A*. *lumbricoides* and *G. lamblia* cause bleeding through lesions formed in the intestinal mucosa as the parasites compete for food, resulting in iron loss.[12] Children infected with *T. trichiura* who develop trichuriasis face the consequences of chronic dysentery (*T. trichiura* dysentery syndrome), IDA and poor growth during heavy parasitic infestations.[13]

Gastro-intestinal parasitic infections can reduce the absorption of micronutrients. Infections with *G*. *lamblia* are associated with decreased zinc[14] and vitamin A absorption.[15] Co-infections with *G. lamblia* and *Enterobius vermicularis* lower serum levels of vitamin B12 in symptomatic patients.[16] Micronutrient deficiencies are also documented to contribute to an increased risk of intestinal parasitic infections. Micronutrient deficiencies including iron[17] and zinc[18] deficiencies have been documented as contributing to an increased risk of IPIs. These findings demonstrate that the consequences of parasitic infections on human nutrition, and the consequences of under-nutrition on parasitic infections, are highly interlinked.

1.2 Intestinal parasitic infections in the global context

Diseases caused by IPIs (helminths and protozoa) remain a significant global threat. Achieving their prevention and control was recently recognised as a priority by the United Nations Millennium Development Goals (MDGs).[19] Neglected parasitic diseases are highly endemic among marginalised populations of low-income countries, favoured for their prevailing poor environmental and sanitary conditions.

Moreover, the expansion of AIDS has resulted in significant changes in the fauna of infectious parasites and in the severity of infections.[20] Various parasites previously considered as sporadic or zoonotic have become causative agents of life threatening diarrhoea in immunocompromised patients.[21]

IPIs are estimated to affect more than two billion people globally,[22] with more than five billion people at high risk of infection.[23] Approximately 39 million disability adjusted life years (DALYs) are attributed to IPIs, representing a substantial economic burden.[24] Among the parasitic Neglected Tropical Diseases (NTDs), infections with worms (helminths) are of particular importance in terms of their global burden,[25] and among these, infections with STHs are the most prevalent chronic human infections.[26] According to recent global estimates, more than 438 million people are infected with hookworm, 819 million people are infected with *A. lumbricoides*, and more than 464 million people are infected with *T. trichiura.*[27] Sub-Saharan Africa has the highest prevalence of infection caused by these parasites. In this area of Africa, approximately 85% of the total NTD burden (affecting a total of 500 million people) results from these specific helminth infections.[28]

Intestinal protozoan infections are also common in humans worldwide, with infections in childhood, pregnancy and AIDS sufferers being of major importance.[3] *E. histolytica*, *G. lamblia*, and Cryptosporidium species are among the most common intestinal protozoan parasites and, diseases caused by these parasites are associated with diarrhoea.

Three morphologically identical species in the genus *Entamoeba* commonly infect humans, namely *E. histolytica*, *Entamoeba dispar*, and *Entamoeba moshkovskii.*[29] *E. histolytica* is the causative agent of amoebiasis, and intestinal amoebiasis is one of the top ten causes of severe diarrhoea in the developing world.[30] Amoebiasis is the third leading cause of death from parasitic diseases worldwide, with an estimated rate of up to one-hundred thousand people per year dying from dysentery, colitis, and extra intestinal complications caused by subsequent infections.[31] *E. histolytica* is estimated to infect approximately 480 million people worldwide,[32] however, these estimations are currently being reassessed due to advancements in molecular testing.[33]

G. lamblia (synonymous: *Giardia duodenalis* or *Giardia intestinalis*) is a ubiquitous enteric protozoan pathogen causing diarrhoeal illnesses in humans worldwide, with an estimated prevalence of 28 million infections annually.[34] The parasite was recently included in the World Health Organisation (WHO) Neglected Disease Initiative.[3] The resulting disease from infection with *G. lamblia*, giardiasis, particularly affects children and immune-deficient individuals. Infection with this parasite appears to be associated with a three-fold increase in the risk of persistent diarrhoea among children in developing countries.[35]

Another related and widely spread enteric protozoan pathogen to infect humans is the Cryptosporidium species. The parasite is a major causal agent of diarrhoea in both developed and developing countries, primarily affecting patients with AIDS and children under five years of age.[36] The recognition and identification of Cryptosporidium as a 'neglected pathogen' in the WHO Neglected Disease Initiative[3] indicates its increasing importance in developing countries as well as a global burden.

Children bear a heavy burden from IPIs, as both the principal sufferers and the source of continued transmission. In poor settings, children are continuously infected and re-infected by chronic intestinal parasites, negatively affecting all aspects of their development.[37] School-aged children in particular are at greater risk for disease caused by infections with STH than any other age group.[25] Approximately 400 million school-aged children are estimated to be infected globally with STHs, schistosomiasis and other flukes. Often these children are infected with multiple species.[38] Approximately one billion school-aged children are estimated to live in areas stable for transmission of at least one STH species.[23]

1.3 Under-nutrition in the global context

The number of under-nourished people worldwide remains unacceptably high. One of the eight MDGs concerns the eradication of extreme poverty and hunger (MDG1). Alleviation of extreme hunger is of a significant importance, not only as the explicit target of MDG 1, but also as an essential condition for reaching other MDGs.[39] The 2015 global nutrition targets of the World Health Assembly aims to reduce childhood stunting by 40%, reduce and maintain the prevalence of wasting below 5%, and halve the rate of anaemia in women of childbearing age.[40] In 2010, under-nutrition affected nearly one billion people globally, with 98% living in developing countries, and approximately 75% in rural areas.[39] In such settings, the two immediate causes of under-nutrition - inadequate dietary intake and infectious diseases - interact in a complex manner and manifest in chronic or acute health and developmental deficiency in children.[41]

1.3.1 Protein-energy under-nutrition

Protein-energy under-nutrition (PEU) is of a significant public health issue worldwide. Stunted, underweight, and wasted children have an increased risk of death from infectious diseases. According to recent estimates, stunting and underweight each account for approximately one million deaths, while approximately 800,000 deaths are attributed to wasting.[42] Universally, growth monitoring is the tool for assessing the nutritional status, health and developmental well-being of children. Anthropometric measurements, usually weight and height in relation to an individual's age, are combined to construct anthropometric indices.[43] These indices can be expressed in terms of z-scores (standard scores) or percentiles which enable comparisons of individuals to a reference population. According to the WHO Child Growth Standards, stunting (height-for-age <–2 SD of the median), under-weight (weight-for-age <–2 SD of the median), and wasting (low weight-for-height <–2 SD of the median) are the most commonly used anthropometric indices to evaluate the nutritional well-being of a child.[44]

Stunting (low height-for-age) is a useful anthropometric measure in children as it elucidates the multiple dimensions of children's health and development.[45] It reflects nutritional deficiencies and illnesses during the early-life period, which are likely to hamper growth and development. In 2012, approximately 162 million children globally under the age of five had stunted growth, and 92% of these children lived in only two continents; Asia (56%) and Africa (36%).[46] Despite some reductions observed in other regions, the burden of childhood stunting has increased in Africa,[47] accounting for more than 56 million children.[42] Causes for stunting extend beyond hunger, and the consequences are wide-ranging, preventing affected communities and nations from achieving their social and economic development goals. Stunting is untreatable, and its prevention demands a combined effort of multiple measures which should emanate from a range of disciplines.[48]

Underweight (low weight-for-age) is an indicator of both acute and chronic under-nutrition; representing a significant health problem in developing countries, where poverty is a strong underlying determinant. Lack of adequate nutritional intake and repeated attacks with infectious diseases contribute to the high prevalence observed in low-income settings.[49] Childhood underweight is the leading cause of global disease burden,[50] and a significant proportion of deaths in young children are related to underweight.[51] Although the global number of underweight cases has decreased, it is argued to be insufficient to meet the MDG and reduce its burden by half by 2015. In 2012, an estimated 99 million children under the age of five worldwide were underweight.[46]

The weight-for-age indicator is unable to distinguish between relative height and body weight, which renders it inadequate for monitoring growth in older children. A complementary indicator, the Body-Mass-Index (BMI)-for-age was therefore recommended for growth monitoring in children beyond pre-school age (5 to 19 years), as it can capture changes in weight and height in school-aged children and adolescents that are respective of their age. Low BMI-for-age is suggested for the assessment of thinness while high BMI-for-age is used for the assessment of obesity.[52]

Wasting (low weight-for-height) is an indicator reflecting weight loss relative to height, due to a short-term loss of muscle mass. It concerns acute under-nutrition resulting from weight loss due to infection and acute food shortage. According to 2012 estimates, approximately 51 million children under five were wasted; approximately 97% of whom lived in Asia (69%) and Africa (28%). An additional 17 million children were severely wasted globally.[46]

1.3.2 Micronutrient deficiencies

Micronutrient deficiencies are significant contributors to the burden of under-nutrition. They are highly prevalent in children in developing countries due to the strong presence of infectious diseases and inadequate food intake. Although the roles of various nutrients during childhood development are highly recognised, the focus of this thesis is confined to the prevalence, risk factors and prevention of anaemia among school-aged children.

Anaemia is a condition in which the number of red blood cells (RBCs) in blood or the concentration of haemoglobin in the RBCs is lower than normal. Anaemia is highly prevalent worldwide. According to WHO estimates, more than 2.6 billion people worldwide are anaemic,[53] and approximately half of the entire anaemia burden is attributed to iron deficiency.[54]

Iron deficiency is the most important contributor to the development of anaemia, and hence IDA and anaemia are used interchangeably. Iron is used for the formation of haemoglobin, and iron-deficient production of RBCs leads to reduced haemoglobin levels. However, depending on the local conditions, causes of anaemia can also vary. Abnormal blood losses due to menstruation and IPIs (such as hookworms, ascaris and schistosomiasis), chronic infections (malaria, tuberculosis and HIV), and deficiencies in other micronutrients (including vitamin A and B12, folate, riboflavin and copper) can also lower haemoglobin concentrations and increase the risk of anaemia.[53]

Children in developing countries are among the most at-risk populations for developing anaemia due to rapid growth and subsequent physiological requirements for iron and other nutrients, combined with the increased risk of infection and poor dietary intake associated with poor living conditions. Anaemic children are reported to have impaired physical and cognitive development[54] while young children are most vulnerable to the long-term effects of anaemia. According to recent data from 187 countries, children under 5 years of age were the only group with an increased prevalence of anaemia in the years 1990 to 2010. Over 47% (293 million) of pre-school and 25% (305 million) of school-aged children are affected by anaemia worldwide. Africa is one of the WHO regions associated with the highest risk of anaemia among children, accounting for approximately 68% of all anaemic pre-school aged children, according to 2008 estimates.[53]

Despite the recent global interest in solving health problems of school-aged children in developing countries, data pertaining to their disease burden are lacking.[55] The multi-factorial nature of health problems in this age group requires an integrated approach to identify the extent of health problems and to address their contributing factors. Simple public health prevention measures against infection need to be identified and integrated into health intervention planning. Knowledge of the infection and nutritional status of children and immunocompromised patients has far-reaching implications for health promotion and could be a valuable input for decision-makers in setting policy priorities and monitoring intervention programs.

1.4 IPIs and under-nutrition rates in the Ethiopian context

1.4.1 Geography of Ethiopia

Ethiopia, identified as the ancient home of mankind, is situated in the Horn of Africa, bordered by Eritrea on the north and north east, Djibouti and Somalia on the east, Kenya on the south, with Sudan and The Republic of South Sudan on the west and south west (Figure1.2). Covering 1,104,300 square kilometres, Ethiopia is the tenth largest country in Africa. The country has wide geographic diversity ranging from the highest peak approximately 4,550 meters above sea level (at Ras Dashen) to the lowest at about 130 meters below sea level (at Dallol in the Afar depression). A large proportion of Ethiopia is categorised as highland lying 1,500 meters above sea level. It has three principal climates, predominantly tropical monsoon, with temperate climate on the plateau and hot in the lowlands.[56,57]

Figure 1.2 Map of Ethiopia, its Regional states and Administrative Councils. (Source: https://www.irishaid.ie/media/irishaid/allwebsitemedia/30whatwedo/ethiopia-map-large-490x401.png)

1.4.2 Demographic situations and governmental structure

Ethiopia is one of the least urbanised countries in the world: only 16.4% of the total population lives in urban areas, with 83.6% living in rural settlements. The majority of the population lives in the highland areas and the main occupation of the rural population is farming. The lowland areas are mostly inhabited by pastoral people, who depend mainly on livestock production. Projections from the 2007 census estimated the total population for the year 2010 to be 79.8 million with an annual population growth rate of 2.6%. The average household size is 4.7 members. Forty four percent of the total population is aged below 15 years, and over half (52%) of the population is in the age group of 15-65 years. The sex ratio between male and female for the country is almost equal (50.5% male and 49.5% female).[56,57].

The country is a federal state with two parliaments: the House of Representatives and House of Federation. The administrative structure comprises nine regional states (Tigray, Afar, Amhara, Oromia, Somali, Benishagul Gumuz, Southern Nations Nationalities and Peoples Region (SNNPR), Gambella, and Harari) and two Administrative Councils (Addis Ababa and Dire Dawa city councils). The regional states and administrative councils are further subdivided into 817 basic decentralised administrative units called woredas, and the woredas are further divided into approximately 15,000 kebeles, the smallest local administrative units in the governance hierarchy.[56,57]

Our studies were conducted in one of the woredas of the Tigray Regional State (Figure 1.3), Kilte Awlaelo. Located in the northernmost of the country, Tigray is bordered by Eritrea in the north, the Amhara region in the south, the Afar region in the east, and Sudan in the west. With an estimated area of 54,569.25 km², the region is administratively divided into seven zones including one special zone, Mekelle - the region's capital city. It has 46 woredas/districts (34 rural and 12 urban) and 763 kebeles (702 rural and 61 urban). According to the projected census of 2007, the region had a total population of 4,806,843 (3,787,667 rural and 1,019,176 urban) in 2010.[58] Figure 1.3 shows the study sites of the Kilte Awalelo woreda included in the thesis.

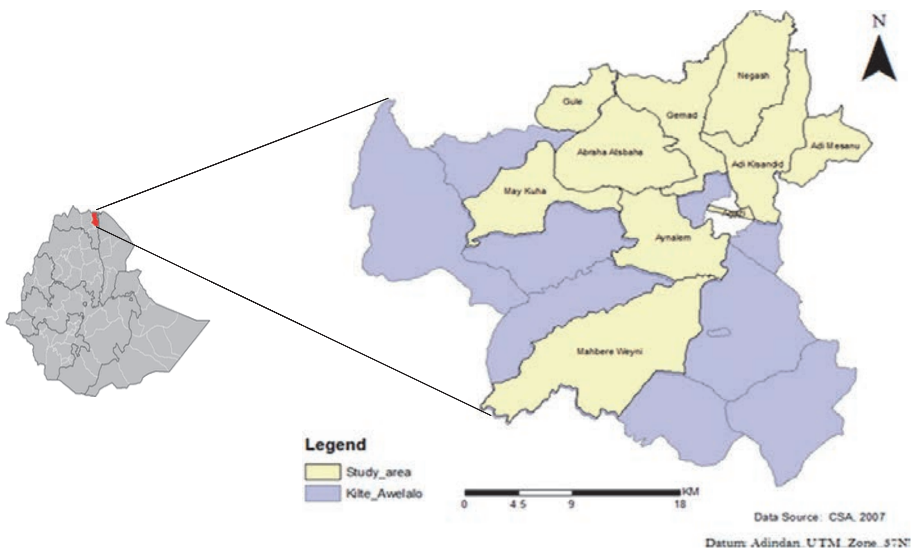

Figure 1.3 Map of Kilte Awlaelo woreda indicating study sites.

1.4.3 Health status of the population of Ethiopia

The health status of the population remains relatively poor despite major progress made in the health sector. Preventable communicable diseases and nutritional disorders are major causes of high morbidity and mortality. The core elements of Ethiopia's health policy involve development of preventive and curative components of health care, and assurance of accessibility for all segments of the population. The government of Ethiopia has devised a number of strategies which provide a framework for improving child health. Human Immunodeficiency Virus (HIV) and AIDS prevention and control is also recognised as a top priority health intervention in the country. Overall, the national HIV prevalence for the year 2009 was 2.3%; with an urban and rural prevalence of 7.7% and 0.9%, respectively.[56-58]

Since 2004, the government of Ethiopia has made a great move to strengthen its health system towards the values of primary health care. A new health care plan, the "Accelerated Expansion of Primary Health Coverage", through comprehensive Health Extension Program (HEP), has been launched as an effective mechanism for shifting health care resources to rural areas where majority of the country's population resides.[59-60]

1.4.4 IPIs and under-nutrition rates in Ethiopia

Infectious diseases, diarrhoea and nutritional disorders are the major causes of death in infancy and childhood in Ethiopia. According to the 2011 Ethiopian Demographic and Health Survey report, 13% of children under the age of five had diarrhoea during the survey period. Nationally, more than two in five children (44%) were anaemic. Similarly, 44% of children under age five were stunted, 29% were underweight and 10% were wasted. Prevalence of anaemia and protein-energy malnutrition was higher among children in rural settings.[57] Ongoing poverty, poor dietary intake and inadequate provision of hygiene facilities make intestinal parasitosis and under-nutrition major public health problems in Ethiopia.

The distribution and prevalence of the various species of intestinal parasites vary from region to region due to differences in environmental, social and geographic factors. Overall, data from recent studies indicate high prevalence of intestinal parasitosis among school children[61,62] and HIV infected patients.[63] Additionally, personal hygiene and sanitation practice were shown to be statistically associated with high parasitic infection prevalence among these groups. Several studies have documented high prevalence of PEU among school children, and PEU was indicated to be significantly associated with intestinal parasitosis.[64,65] Although most of the data available involves pre-school children, some studies also indicate the presence of anaemia among school-aged children.[66]

Like any other region in the country, the commonest health problems in Tigray are communicable diseases. According to the 2004 Regional Health Bureau report, the commonest child health problems in the region were pneumonia, diarrhoea and malaria. Child health problems in the region are, among others, attributable to poor personal hygiene and environmental sanitation and are aggravated by high rates of under-nutrition. At regional level, diseases due helminthiasis and other intestinal parasites are amongst the top ten causes of morbidity among out patients.[67]

1.5 Aims of the research project

The main aims of this thesis are to determine the magnitude and associated risk factors for intestinal parasitosis and under-nutrition among vulnerable populations (school-aged children and HIV/AIDS patients); to empirically assess the impact of simple public health interventions (hand-washing with soap and finger nail clipping) on the prevention of intestinal parasite re-infection rates and anaemia among school-aged children; to describe the associations between parasitic infection, anaemia and diarrhoea in children, and to evaluate the intervention effects on intestinal parasite re-infection rates in different children defined by different baseline characteristics.

1.5.1 Research questions

Based on the above mentioned aims, the following research questions were formulated:

1. What is the prevalence and what are the associated risk factors for intestinal parasitosis in adult HIV/AIDS patients in Ethiopia?
2. What is the magnitude of intestinal parasitosis and under-nutrition among school children in impoverished areas of Northern Ethiopia, and what are the associated risk factors?
3. Do regular hand-washing with soap and weekly finger nail clipping significantly prevent intestinal parasite re-infection and reduce anaemia prevalence in school-aged children in endemic areas?
4. How are IPIs, anaemia and diarrhoea associated among affected children; and do the effects of intervention vary among children with different baseline demographic and health characteristics?

1.5.2 Outline of the thesis

Chapter 2 describes the prevalence and associated risk factors for IPIs in HIV/AIDS patients. In this chapter we include results from our research into the magnitude and associated risk factors for IPIs in anti-retroviral treated adult AIDS patients. In Chapter 3 we report on the magnitude and associated risk factors for IPIs, anaemia and PEU among rural school children. School children from 12 rural primary schools were assessed for IPIs and malnutrition, and associated risk factors. Findings from the studies presented in this chapter led to the research hypothesis of the randomised controlled trial (RCT) outlined in Chapter 4. Chapter 4 describes the impact of simple public health interventions on the prevention of intestinal parasite re-infection and anaemia in rural school-aged children. In this RCT, we investigated the effects of regular hand-washing with soap and weekly nail clipping on infection prevention and anaemia prevalence in school-aged children living in an impoverished rural area. In Chapter 5 we assess associations between parasitic infection, anaemia and diarrhoea in children, and the likelihood of these health problems in association with demographic characteristics, history of IPIs and under-nutrition among the study participants. We also explore the impact of the interventions in children with different baseline characteristics. Data from a previous RCT (Chapter 4) was used to conduct the subgroup and association assessments. Chapter 6 outlines the methodological considerations of the studies presented in this thesis, and explores implications for practice and further research. Chapter 7 concludes with brief summary of the overall major findings, and the conclusions of each study presented in this thesis.

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Chapter 2

Risk factors for intestinal parasitosis among antiretroviral treated HIV/AIDS patients in Ethiopia

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Abstract

A cross-sectional survey was conducted to determine the risk factors associated with intestinal parasitosis in HIV/AIDS patients receiving antiretroviral treatment. Sociodemographic information was collected and faecal samples were analysed from 384 randomly selected patients under antiretroviral treatment. Data on CD4+ T-cell counts and World Health Organization clinical staging were obtained from medical records at the Adigrat hospital, Ethiopia. The overall prevalence of intestinal parasitosis was 56% (95% confidence interval (CI): 51% to 61%). No opportunistic intestinal parasites and *Schistosoma haematobium* eggs were detected. Unavailability of latrine and lack of hand washing with soap were associated with *Entamoeba histolytica*/*dispar* (adjusted odds ratio (AOR), 2.75; 95% CI: 1.77 to 4.27 and AOR, 2.67: 95% CI: 1.60 to 4.44, respectively) and *Giardia lamblia* (AOR, 2.08; 95% CI: 1.08 to 3.99 and AOR, 2.46; 95% CI: 1.06 to 5.75, respectively) infections. Intestinal parasitosis was significantly associated with low CD4+ T-cell count ($p=0.002$). In contrast, intestinal parasitic infections were not associated ($p>0.05$) with the World Health Organization disease staging. In summary, poor personal hygiene and sanitation practice contributed to the high prevalence of intestinal parasitosis. Routine diagnosis for intestinal parasitic infections should be performed in patients attending antiretroviral treatment clinics.

Introduction

Intestinal parasitosis[1] and Human Immunodeficiency Virus (HIV) infection[2] are highly endemic in resource-poor settings. Approximately one-third of the almost three billion people living in marginalised regions of the world are infected with one or more helminth parasites.[1] Over 60 percent of the people living with HIV globally are estimated to live in sub-Saharan Africa.[3] Like many other developing countries, Ethiopia is highly affected by the pandemic; with nearly 800,000 people living with HIV.[4] Similarly, intestinal parasitic infections (IPIs) are also widely distributed among the population,[5-7] due to lack of safe drinking water, and poor hygiene and sanitary conditions.

The expansion of the HIV/AIDS pandemic has resulted in significant changes in the fauna of intestinal parasites. More importantly, the epidemiology and outcome of diseases caused by opportunistic parasites has significantly changed.[8,9] Various intestinal parasites, previously considered to be sporadic or zoonotic, have become common causative agents of life-threatening diarrhoea.[10,11]

Infection with intestinal parasites causes chronic immune activation and contributes to the rapid progression of HIV into AIDS.[12,13] Significant correlations between number of excreted eggs and plasma viral load have been documented, with subsequent reduction of viral load following eradication of IPIs.[14]

In Ethiopia, previous studies have shown high prevalence of IPIs among HIV/AIDS patients.[15-17] However, information regarding the magnitude and risk factors associated with IPIs among patients receiving antiretroviral treatment (ART) is still very limited. The purpose of this study, therefore, was to determine the prevalence and associated risk for IPIs among HIV/AIDS patients receiving ART at Adigrat hospital in northern Ethiopia.

Methods

Study design and study population

The cross-sectional study was carried out among patients receiving ART at Adigrat hospital in northern Ethiopia. The study area was selected by purposive sampling. Purposive sampling was used due to the relatively large number of HIV/AIDS patients receiving ART in this location during the study period and for the location's relative accessibility. The study population consisted of 384 randomly selected patients aged 17-64 years visiting the ART clinic for treatment. Of those randomly selected, only patients who were not treated with anti-parasitic drugs and not involved in any de-worming program within two weeks before sample collection were included in the study. The selected subjects were invited to participate in the study after written informed consent was obtained.

Data collection

Socio-demographic information

Structured questionnaires were administered by trained medical professionals working in the clinic in the local language to generate information on personal bio-data and other sociodemographic and socioeconomic information. Daily close supervision (spot checks, re-interviewing and thorough scrutiny of completed questionnaires) was conducted by field supervisors deployed with the data collectors.

Parasitological examination

From each subject, approximately 10 grams of fresh stool and 10 mL of midday urine specimens were collected in clean labelled containers and were analysed by well-trained laboratory technologists. Sub-samples of stool and urine, comprising 10 percent of total samples, were re-examined for quality control purposes. A subject was classified as infected if an IPI was detected by any of the methods used.

Direct microscopy (Wet mount)

A direct wet mount of stool specimen in normal saline (0.85% NaCl solution) was freshly prepared and analysed under light microscope (100x and 400x magnifications) within less than one hour after preparation for the detection of ova, larvae, trophozoites and cysts of intestinal parasites.

Concentration method

Formalin-ethyl acetate method

A portion of each fresh stool sample was processed using formalin ethyl acetate concentration technique.[18] Briefly, approximately 4 grams of stool specimen was mixed with 10 ml of 10% formalin and sieved with double layered gauze in to a conical centrifuge test tube. Ten percent formalin was then added, and centrifuged for 10 minutes at 500 x g. Supernatant was discarded and sediment re-suspended with formalin. Two ml of ethyl acetate was added and centrifuged for 10 minutes, at 500 x g, after vigorous hand shaking. The supernatant was discarded and the sediment analysed for the presence of ova and/or parasites under the light microscope, at magnifications of 100x and 400x.

Kato-Katz method

Duplicate Kato-Katz thick smear slides were prepared for each stool specimen by one experienced laboratory technologist to avoid inter-observer bias. The Kato-Katz smears were prepared and analysed by placing a small amount of faecal specimen on a clean plastic sheet. A piece of a mesh was then pressed on top, and the upper surface of the mesh was scraped using a flat-sided spatula to collect the sieved faeces. A template was placed on a slide and the sieved faeces were added with the spatula to completely fill the hole in the template. The template was carefully removed and the faecal material was covered with a pre-soaked cellophane strip. The slide was then inverted and the faecal sample firmly pressed against another slide to evenly spread the specimen. Prepared slides were placed on the bench with cellophane facing upwards, enabling the evaporation of water while glycerol cleared the faeces. Prepared slides were microscopically analysed within 30 minutes for identification of hookworm eggs, and after 72 hours for the identification of ova of other helminths.[19] The total number of eggs detected on each slide was counted and the number of eggs per gram of faeces (epg) was calculated to determine egg burden using the conversion factor.

Modified Ziehl Neelsen staining method

Detection of opportunistic protozoan parasites like *Cryptosporidium* and *Isospora* oocysts was achieved using the modified Ziehl-Neelsen staining technique.[20] Briefly, a concentrated smear was prepared from fresh stool on a slide, and fixed with methanol after being air dried. The smear was immersed in cold carbol fuchsin for 15 minutes and rinsed with water. Preparation was decolourised with 1% acid alcohol (5 ml of 37% hydrochloric acid and 495 ml of 70% ethanol) for 15 seconds. After rinsing again in tap water, the slide was counter-stained with methylene blue for 30 seconds. The smear was examined microscopically for oocysts using a low-power magnification and oil immersion objectives after being air dried.

Urine sedimentation test

Ten ml urine specimens, collected between 10:00am and 2:00pm in clean containers, were transferred to a conical tube and centrifuged at 500 x g. Supernatant was discarded and the sediment examined microscopically (10x objective) for the detection of *S. haematobium* ova.[20]

Immunological profile

Data on CD4+ T-cell counts obtained during the study period and WHO clinical staging of the study participants were acquired from medical records at the hospital.

Statistical analysis

Data were entered in an Excel spreadsheet, cleaned and anonymised. Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 16 (Chicago, USA). Descriptive statistics were used to analyse the prevalence of the outcome variables. Chi square and logistic regression tests were used to investigate associations between potential variables or risk factors by odds ratio (OR) and 95% confidence interval (CI). Independent sample t test was used for comparison of means. Significance was set at $p<0.05$.

Ethical considerations

Ethical clearance was obtained from the Ethical Review Board of the College of Health Sciences, Mekelle University, Ethiopia (ERC0032/2011). Written permission to conduct the study was sought from the Regional Health Bureau and the hospital. Written consent was obtained from study participants. Participants diagnosed positive for IPIs were treated with standard regimen.[21]

Results

Of the 384 cases selected for the study, 372 (97%) were able to provide stool and urine specimens. Analysis was conducted on the 372 subjects who were able to provide specimens. Males comprised 40% (n=150) of study participants. The median age of the participants was 34 years (range 17-64 years). Table 2.1 describes the prevalence of parasitic infections (56%, 95% CI: 51% to 61%).

E. histolytica/dispar (overall prevalence of 40%) was the most commonly detected parasite, followed by *G. lamblia* and Taenia species. Except for Taenia species infection ($p=0.015$), no significant difference ($p>0.05$) was observed on the prevalence of intestinal parasitosis among male and female participants (Table 2.1). Prevalence of multiple parasitic infections was approximately 12% (45/372) and *E. histolytica/dispar* and *G. lamblia* were the most common parasite combinations detected in patients with multiple parasitic infections. No opportunistic intestinal parasites or *S. haematobium* eggs were detected.

Egg concentrations of helminthic infections were of light intensity (less than 1,999 eggs per gram of stool (epg) for hookworms, and less than 4,999 epg for *Ascaris lumbricoides*).[22] The arithmetic mean egg counts for the identified soil-transmitted helminths were 75 epg for hookworm (range: 24 to 96 epg) and 40 epg for *A. lumbricoides* (range: 24 to 48 epg). Mean CD4+T-cell count was 302cells/mm^3. Intestinal parsitosis was significantly associated with decreased CD4+T-cell count (p=0.002). In contrast, there was no association (p>0.05) between intestinal parasitosis and WHO disease staging (Data not shown).

Table 2.1 Prevalence of intestinal parasitosis among HIV/AIDS patients under anti retroviral treatment, Ethiopia (n=372).

Dependent variables	Male Number (%) (n=146)	Female Number (%) (n=226)	Total Number (%) (n=372)	p-value
Intestinal Parasitic Infections				
Protozoa				
Entamoeba histolytica/dispar	61 (42)	86 (38)	147 (40)	0.473
Giardia lamblia	18 (12)	29 (13)	47 (13)	0.887
Nematodes				
Hookworm	3 (2)	5 (2)	8 (2)	0.919
Ascaris lumbricoides	2 (1)	1 (0.4)	3 (0.8)	0.329
Enterobius vermicularis	2 (1)	1 (0.4)	3 (0.8)	0.329
Strongyloides stercoralis	0 (0)	1 (0.4)	1 (0.2)	0.421
Cestodes				
Hymenolepis nana	6 (4)	5 (2)	11 (3)	0.292
Taenia species	18 (12)	12 (5)	30 (8)	0.015[a]
Total parasitosis	89 (61)	119 (53)	208 (56)	0.115
Multi parasitic infections	22 (15)	23 (10)	45 (12)	0.158

[a] statistically significant at p<0.05

Univariate and multivariate logistic regression analysis (adjusted for age, gender, level of education and family size) results for IPIs are shown in Table 2.2. Our findings revealed a strong positive association between poor personal hygiene habits and intestinal parasitosis (Table 2.2). Overall, we found a relevant pattern of associations between intestinal parasitosis and poor hygiene and sanitation practices. Unavailability of latrine and lack of hand washing with soap at critical times were significantly associated with *E. histolytica/dispar* (adjusted odds ratio [AOR], 2.75: 95% CI: 1.77 to 4.27 and AOR, 2.67; 95% CI: 1.60 to 4.44, respectively) and *G. lamblia* (AOR, 2.08, 95% CI: 1.08 to 3.99 and AOR, 2.46; 95% CI: 1.06 to 5.75, respectively) infections. Female participants were less likely to be infected with human Taenia parasites (AOR, 0.34; 95% CI: 0.15 to 0.74) (Table 2.2).

Table 2.2 Associations of Intestinal parasitic infections among HIV/AIDS patients under anti retroviral treatment, Ethiopia (n=372).

	n	*E. histolytica/dispar* COR (CI) AOR (CI)[a]	%	*G. lamblia* COR (CI) AOR (CI)[a]	%	Taenia sp. COR (CI) AOR (CI)[a]	%	*H. nana* COR (CI) AOR (CI)[a]	%
Age									
<34	193	1	34	1	10	1	9	1	4
≥34	179	1.53 (1.01, 2.33)[b]	43	1.44 (0.78, 2.67)	14	0.72 (0.34, 1.55)	7	0.63 (0.18, 2.18)	2
		1.38 (0.88, 2.16)		1.38 (0.88, 2.16)		0.66 (0.29, 1.50)		0.47 (0.12, 1.83)	
Gender									
Male	145	1	41	1	12	1	12	1	4
Female	227	0.86 (0.56, 1.31)	38	1.05 (0.56, 1.96)	13	0.40 (0.19, 0.86)[b]	5	0.53 (0.16, 1.76)	2
		0.97 (0.62, 1.52)		1.13 (0.59, 2.18)		0.34 (0.15, 0.74)[b]		0.53 (0.15, 1.88)	
Family Size									
<3	156	1	46	1	13	1	13	1	3
≥3	216	1.63 (1.07, 2.48)[b]	33	1.81 (0.98, 3.35)	12	0.56 (0.25, 1.25)	4	1.65 (0.49, 5.51)	3
		1.50 (0.96, 2.33)		1.50 (0.96, 2.33)		0.55 (0.23, 1.29)		2.13 (0.59, 7.69)	
Presence of latrine									
Yes	182	1	30	1	10	1	7	1	2
No	190	2.84 (1.85,4.38)[b]	46	2.21(1.16, 4.19)[b]	14	1.39 (0.65, 2.94)	7	1.84 (0.53, 6.39)	4
		2.75 (1.77, 4.27)[b]		2.08 (1.08, 3.99)[b]		1.44 (0.67, 3.10)		2.01 (0.43, 7.12)	
Hand washing with soap									
Yes	108	1	27	1	6	1	9	1	4
No	264	2.77 (1.68, 4.58)[b]	44	2.65 (1.15, 6.12)[b]	15	0.83 (0.37, 1.83)	7	1.92 (0.41, 9.04)	3
		2.67 (1.60, 4.44)[b]		2.46 (1.06, 5.75)[b]		0.85 (0.38, 1.92)		1.01 (0.42, 9.70)	
Family with diarrhoea									
No	309	1	38	1	12	1	8	1	3
Yes	63	1.18 (0.68, 2.05)	41	1.01 (0.45, 2.27)	12	1.25 (0.49, 3.20)	10	0.48 (0.06, 3.84)	2
		1.12 (0.64, 1.92)		1.10 (0.48, 2.51)		1.40 (0.53, 3.65)		0.52 (0.06, 4.20)	
Pipeline water source									
Yes	253	1	37	1	12	1	7	1	3
No	119	1.04 (0.67, 1.62)	40	1.19 (0.63, 2.25)	14	1.64 (0.77, 3.49)	10	1.18 (0.34, 4.10)	3
		1.01 (0.65, 1.59)		1.12 (0.58, 2.14)		1.77 (0.82, 3.84)		1.23 (0.36, 4.51)	

E. histolytica/dispar: *Entamoeba histolytica* and/or *Entamoeba dispar*; *G. lamblia: Giardia lamblia;* COR: Crude odds ratio as computed by logistic regression model; *H. nana; Hymenolepis nana*; AOR: adjusted odds ratio as computed by logistic regression model; Taenia sp.: Taenia species. [a] adjusted for age, gender, education level and family size. [b] statistically significant at 0.05.

Discussion

Our findings revealed widespread prevalence of intestinal parasitosis among ART patients. Only extracellular parasites were identified. *E. histolytica/dispar* and *G. lamblia* were the most prevalent parasites indentified among the study group. IPIs and individual hygiene and sanitation practices were very clearly related. Absence of latrine and hand washing with soap contributed to the higher proportion of IPIs, which suggests the need to address personal hygiene and sanitation practices among HIV/AIDS patients.

The overall prevalence of IPIs (56%) in our data was higher than reports from different studies conducted in the central (35.4%),[23] north-eastern (17.6%)[24] and south-western (39.6%)[25] regions of the country, as well as Kenya (50.9%),[26] Ghana (35%),[27] Nigeria (5.3%)[28] and the Democratic Republic of Congo (15.4%).[29] Variations in geographical locations, socioeconomic conditions and cultural practices of the population under consideration might explain the differences in findings among the studies. The methods employed for stool examination and the time of the study may also have contributed to the differences. High prevalence of IPIs among the study participants may call for better follow-up through laboratory tests and more comprehensive actions by the patients themselves in adopting prevention measures against IPIs.

In agreement with other studies, opportunistic coccidian parasitic infections were not detected in the present study.[23,28,30] ARTs have been documented to improve immune status,[31,32] thereby preventing the occurrence of opportunistic parasitic infections.[30-32] This may explain the absence of opportunistic parasites in this study. The significantly lower prevalence of Taenia parasitic infection among female patients might be related to lower consumption of raw or undercooked meat among this gender group.

Non-opportunistic parasites, including the intestinal parasites *E. histolytica/dispar and G. lamblia*, are frequently encountered in resource-limited countries.[33] Diarrhoea is a common clinical symptom associated with infection by these parasites.[34] Infection with these organisms might therefore contribute to the morbidity among HIV/AIDS patients, thus early detection and treatment of infection is important to improve the quality of life of patients under ART. Associations between poor hygiene, sanitation practices and intestinal parasitosis are well-documented.[35,36] The faecal-oral route is the most common method of transmission, through contaminated hands and fingers.[33]

A major strength of this study was the random selection of study participants. As the study population was randomised, generalisations may be made to other populations with HIV/AIDS and undergoing ART. A limitation of the study was its cross-sectional nature, making any inference on causal relationship among variables impossible. As a result, the study could only generate a hypothesis about the possible role of certain independent variables on the infection status of these patients.

In conclusion, our results emphasise the need for increased personal hygiene and sanitation practices which would improve the health of HIV-infected patients undergoing ART, such as increased access to clean water and soap for hand washing, access to latrines to restrict contamination, and hygiene-related education. Stool examination should be routinely performed in the follow-up of patients attending ART clinics in order to optimise treatment and to implement other preventive measures.

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Chapter 3

Risk factors for intestinal parasitosis, anaemia, and malnutrition among school children in Ethiopia

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Abstract

Research on associated risk factors for IPIs and malnutrition in various geographic regions is needed for the development of appropriate control strategies. The aim of this study was to determine risk factors associated with IPIs, anaemia, and malnutrition in school children living in urban and rural areas of northern Ethiopia. Six hundred school children aged 6-15 years were randomly selected in a cross-sectional survey from 12 primary schools. Sociodemographic and anthropometric data were collected. Faecal samples were examined using direct, concentration, and the Kato-Katz methods. Urine specimens were analysed for *Schistosoma haematobium* ova. Haemoglobin was measured using a HemoCue spectrometer. The overall prevalence of intestinal parasitosis was 72% (95% confidence interval (CI): 66-76%). The prevalence of anaemia, stunting, and thinness were 11% (95% CI: 8-13%), 35% (95% CI: 31-38%), and 34% (95% CI: 30-38%) respectively. Poor personal hygiene habits were generally associated with anaemia and nutritional deficiency, given as a low BMI. Multivariate logistic regression models associated *Schistosoma mansoni* infection with boys, who were also more likely to be malnourished. Hookworm infection was associated with anaemia and unhygienic fingernails.

Access to clean water and latrines, together with hygiene and sanitation communication activities, could improve the health of children in Ethiopia. The use of mobile phone applications in demographic data collection proved to be successful. The potential advantage offered by this technology for parasitological field surveys merits further investigation.

Introduction

IPIs,[1] anaemia, and malnutrition are highly endemic in resource-limited regions.[2,3] School-aged children are at greater risk for disease than any other age group as they are particularly susceptible to parasitic infections.[1,4] In developing countries, 12% of the global disease burden due to intestinal worms is estimated to occur in children aged 5-14 years.[5]

Human gastro-intestinal parasites are associated with an increased risk for childhood malnutrition and growth deficits.[6,7] Parasitic diseases such as helminthiasis result in malnutrition[8] through mechanisms which decrease food intake and nutrient absorption, increase metabolic requirements, and cause direct nutrient losses.[9] Poor health also results in deficits in cognitive development and educational achievements.[3,10]

Many health programmes in developing countries had previously not paid enough attention to improving the health of school-aged children.[11] More recently, however, commitment has strengthened to control IPIs and to improve the health and development of young children.[12] To be effective, interventions aimed at reducing the effects of infection and malnutrition must be based on a proper assessment of the current situation. In this study, we investigated the prevalence and associated risk factors for intestinal parasitosis, anaemia and malnutrition in school children in urban and rural areas of northern Ethiopia. As with other resource-poor areas in the country, lack of access to improved sanitation facilities and poor hygiene behaviour are common in the study area. IPIs are amongst the top ten causes of morbidity following acute upper respiratory tract infection, pneumonia, and diarrhoea.[13] No data on the nutritional status of school children is available, though a study conducted on adolescent girls from the area revealed high prevalence of malnutrition.[14]

Methods

Study design and study population

The cross-sectional study was carried out in October 2010 using 12 primary schools in northern Ethiopia. The study area is approximately 1010 km^2 at an altitude of 1900-2300 m above sea level. The area has a semi-arid climate typical of the region, characterised by inadequate and erratic rainfall, mainly between June and September. Subsistence farming is common among the population.

The study schools were selected by purposive sampling as they were part of the Demographic and Health Surveillance (DHS) site established by the College of Health Sciences of Mekelle University. We incorporated all schools that offered education for grades 1-8 in the DHS districts. Schools in towns with municipal administration were considered as urban, while schools in areas without municipal administration and where the predominant type of economic activity is agricultural were considered as rural.

Six hundred school children, aged between 6-15 years, of 18,628 currently enrolled were selected by a systematic random sampling technique using school rosters as a sampling frame. To obtain the exact ages of the children, the reported age of a child was cross-checked using school records, baptism certificates, local calendars, and information from parents. The selected school children were invited to participate in the study after obtaining a written informed consent from parents or guardians.

Data collection

Socio-demographic information

Demographic and socioeconomic data were collected using an advanced mobile phone application (http://www.episurveyor.org). Structured questionnaires were uploaded onto smart phone (Nokia E71) using Datadyen Episurveyor software. To reduce input errors, simple data collection forms were developed and phones were programmed to prevent progression without answering the current question.

Two separate questionnaires (child and household) were administered by the investigators in a local language to generate information on personal bio-data and other sociodemographic and socioeconomic information. The majority of sociodemographic data were collected by asking parents or guardians. Some variables, including hand hygiene and cleanliness of fingernails, were studied by observation. Physical cleanliness of hands was assessed by checking the palms, fingertips, finger pads, and backs of the hands. Hands were coded as unclean if any dirt was visible, and clean if there was no visible dirt. Fingernails which were clean and trimmed were coded as clean, and untrimmed nails with accumulated dirt were coded as unclean. To ensure uniform understanding amongst all data collectors, in-house training was conducted using role play and subsequent pre-testing in the field prior to actual data collection. Daily close supervision (spot checks, re-interviewing and thorough examination of completed questionnaires) was conducted by the field supervisors deployed with the data collectors.

Parasitological examination

From each subject, approximately 10 g of fresh stool and 10 ml of fresh midday urine specimens were collected in clean, labelled containers. Stool specimens were analysed by well-trained laboratory technologists using direct saline wet mount, formalin ethyl acetate concentration technique,[15] and the Kato-Katz technique (thick smear 41.7 mg).[16] Duplicate Kato slides were prepared for each stool specimen by an experienced laboratory technologist. The Kato and wet mount preparations were analysed within one hour of preparation to detect hookworm eggs and protozoan trophozoites (*Entamoeba histolytica/Entamoeba dispar* and *Giardia lamblia*), respectively. The remaining stool specimens were transported in screw-capped cups with 10% formalin to the laboratory and examined using the concentration method within 2-8 hours after collection. Kato preparations were re-examined after 72 hours for the presence of helminth ova. The total number of eggs detected on each slide was counted and the number of eggs per gram of faeces (epg) was calculated to determine worm burden using a conversion factor of 24, provided with the kit. A child was classified as infected if a positive result was detected by any of the methods used.

Urine specimens screened positive for microhaematuria with reagent test strips and those with gross haematuria were subjected to microscopic diagnosis of *S. haematobium* ova using the sedimentation method.[17] Ten per cent sub-samples of stool smears were re-examined for quality control purposes.

Haemoglobin survey

Haemoglobin concentration was determined in finger prick blood using the HemoCue analyser on site (HemoCue Hb 201+, Sweden).[18] Brief training on the machine operation was provided to the technicians collecting and analysing blood samples before the actual data collection period. The machines were checked on a daily basis using reference microcuvettes as indicated by the manufacturer. Haemoglobin readings were adjusted for altitude. Anaemia was defined for respective age and gender groups based on WHO cut-off values.[19]

Anthropometry

Anthropometric data were collected by recording age, weight, and height of study participants. A portable weight scale was used to measure weight (to the nearest 0.1 kg) and a locally made stadiometer with sliding headpiece was used to measure height (to the nearest 0.1 cm). Each child was weighed with minimal clothing and barefooted. The weighing scale was calibrated using standard calibration weights of 2 kg iron bars. Measurements of weight and height were taken twice and the average recorded.

Anthropometric measurements were converted into height-for-age and body mass index (BMI)-for-age Z-scores using WHO AnthroPlus (version 1.0.4). Children below -2Z-scores for height-for-age and BMI-for-age were classified as stunted and thin, respectively.

Statistical analysis

Data were entered into Excel and anonymised at Mekelle University with additional data cleansing at Alcala University, Spain. Statistical analysis was conducted using Statistical Package for Social Sciences (SPSS) version 16 (Chicago, USA). Descriptive statistics were used to analyse the prevalence of the outcome variables. Chi-square, student's t test, and logistic regression tests were used to investigate associations among potential variables or risk factors by odds ratio (OR) and 95% confidence interval (CI). Statistical significance was set at $p<0.05$.

Ethical considerations

Ethical clearance was obtained from the institutional Ethical Review Boards of the College of Health Sciences, Mekelle University, Ethiopia and Alcala University, Spain. Written permission to conduct the study was sought from the Regional Health Bureau and local Health and Education Offices. Written consent was obtained from parents or guardians of the children. Children who were diagnosed positive for IPIs were treated with standard regimen.[20]

Results

Of the 600 school children selected for the study, 583 (97.2%) were able to provide stool specimens and 525 (87.5%) provided blood specimens. Anthropometric measurements were made on 587 (97.8%) children. Approximately 48% (n=288) of the study participants were male and the mean age was 11.3 years (SD=2.5). Table 3.1 describes the prevalence of parasitic infections (72%, 95% CI: 66 to 76%), anaemia (11%, 95% CI: 8 to 13%), stunting (35%, 95% CI: 31 to 38%), and thinness (34%, 95% CI: 30 to 38%) in this population. We also present the differences between urban and rural areas. *E. histolytica/dispar* proved to be the most commonly detected parasite (overall prevalence of 39%) and was significantly higher in children from rural areas. Hookworm showed a distinct focal nature of distribution, restricted to a few sites in rural areas.

Anaemia prevalence in the study group was of a mild public health importance, and although not statistically significant, was slightly higher in rural areas (12%) than urban (8%). The prevalence of malnutrition was similar across urban and rural schools; approximately 20% of children were classified as both stunted and thin. Children infected with up to six parasite species were identified. The prevalence of multiple parasitic infections was approximately 27% (156/583). *E. histolytica/dispar*, *Hymenolepis nana* and *S. mansoni* were the most common parasite combinations detected in multiple parasitic infections. No *S. haematobium* eggs were detected.

Egg concentrations of helminthic infections were of light intensity.[21] The arithmetic mean egg counts for the most common soil-transmitted helminths and schistosomiasis were: 75 epg for hookworm (range: 24-330 epg), 52 epg for *Ascaris lumbricoides* (range: 24-132 epg), and 75 epg for *S. mansoni* infection (range: 24-720 epg). Mean haemoglobin reading in our survey was 132 g/l (SD=1.2g/l). No cases of severe anaemia (haemoglobin <70.0 g/l) were detected. Mean haemoglobin concentration among anaemic subjects was 113 g/l (SD=1.3 g/l). Further, there was no significant association (p>0.05) between individual hygiene and sanitation practice and haemoglobin readings among anaemic children (data not shown). There was no association between anaemia and stunting (p=0.209) or thinness (p=0.640) (data not shown). Similarly, there was no significant association between IPIs and stunting (p=0.404) or low BMI (p=0.864) (data not shown).

Table 3.1 Urban versus rural prevalence of intestinal parasitosis, anaemia, and malnutrition among school children, northern Ethiopia.

Dependent variables	Urban No. (%) (n = 149)	Rural No. (%) (n = 451)	Total No. (%) (n = 600)	p-value
Intestinal Parasitic Infections *				
E. histolytica/dispar	44 (31)	183 (41)	227 (39)	0.021
G. lamblia	14 (10)	45 (10)	59 (10)	0.880
Nematodes				
Hookworm	0 (0)	31 (7)	31 (5)	0.001
Ascaris lumbricoides	18 (12)	10 (2)	28 (5)	<0.0001
Enterobius vermicularis	13 (9)	73 (16)	86 (15)	0.028
Strongyloides stercolaris	0 (0)	5 (1)	5 (0.9)	0.200
Trichuris trichiura	1 (0.7)	0 (0)	1 (0.2)	0.079
Trematodes				
Schistosoma mansoni	21 (15)	59 (13)	80 (14)	0.700
Cestodes				
Hymenolepis nana	30 (21)	95 (22)	125 (21)	0.877
Taenia sp.	3 (2)	1 (0.2)	4 (0.7)	0.019
Total parasitosis	100 (70)	321 (73)	421 (72)	0.483
Anaemia†	11 (8)	46 (12)	57 (11)	0.228
Stunting‡	46 (32)	157 (35)	203 (35)	0.529
Thinness‡	47 (33)	153 (34)	200 (34)	0.714
Stunting and thinness‡	30 (21)	88 (23)	118 (20)	0.801

* Stool specimens were collected from 143 (urban), 440 (rural) and 583 (total) children. † Blood specimens were collected from 136 (urban), 389 (rural) and 525 (total) children. ‡ Anthropometric measurements were taken from 142 (urban), 445 (rural) and 587 (total) children.

Exploratory univariate and multivariate logistic regression analysis (adjusted for age and sex) results for IPIs are shown in Table 3.2. Overall we found no relevant pattern of association between intestinal parasitosis and drinking water source, personal hygiene, or sanitation practices. We found positive associations between *E. histolytica/dispar* and drinking water from hand pump sources. In addition, children who defecate around bushes and homesteads and those with unclean fingernails were more likely to be infected with hookworm. Infection with *S. mansoni* was significantly higher in male children.

Our findings revealed somewhat stronger positive associations of poor personal hygiene habits with anaemia and malnutrition (Table 3.3). *E. histolytica*, hookworm and *S. mansoni* were incorporated in the analysis because of their high prevalence and nutritional significance, respectively.

Children who did not use latrine and had unclean hands and fingernails were more often anaemic and malnourished. Males were more likely to be anaemic and malnourished than females. Infection with the hookworm, *E. histolytica*/*dispar*, and having unclean hands were significantly associated with anaemia. Unclean hands were also associated with thinness. Little difference was observed between the crude and adjusted odds ratios (Tables 3.2 and 3.3), indicating that the observed associations were not affected by age and gender of the child.

Table 3.2 Associations of intestinal parasitic infections among school children, Ethiopia.

		E. histolytica/dispar COR (CI) AOR (CI)§	%	Hookworm COR (CI) AOR (CI)§	%	*S. mansoni* COR (CI) AOR (CI)§	%	*E. vermicularis* COR (CI) AOR (CI)§	%	*H. nana* COR (CI) AOR (CI) §	%
Age											
6-9	169	1	34	1	4	1	9	1	16	1	31
10-15	431	1.50 (1.02, 2.21)*	43	2.01 (0.76, 5.33)	6	1.74 (0.96, 3.15)	17	0.90 (0.54, 1.49)	14	0.47 (0.31, 0.71)*	15
		1.03 (0.53, 1.98)*		2.86 (0.68, 12.07)		0.99 (0.37, 2.65)		1.30 (0.55, 3.10)		0.73 (0.34, 1.56)	
Gender											
Male	288	1	37	1	5	1	18	1	14	1	23
Female	312	1.16 (0.83, 1.61)	41	1.30 (0.62, 2.70)	6	0.48 (0.29, 0.78)*	10	1.14 (0.72, 1.80)	16	0.85 (0.57, 1.26)	20
		1.15 (0.82, 1.61)		1.26 (0.60, 2.65)		0.47 (0.29, 0.76)*		1.15 (0.72, 1.83)		0.86 (0.57, 1.28)	
Family Size											
≤ 7	372	1	42	1	8	1	15	1	17	1	19
> 7	228	1.28 (0.74, 2.24)	48	0.57 (0.17, 1.84)	5	0.93 (0.42, 2.06)	14	1.32 (0.65, 2.67)	21	1.39 (0.71, 2.71)	25
		1.29 (0.73, 2.30)		0.48 (0.14, 1.67)		0.87 (0.38, 2.01)		1.44 (0.69, 3.02)		1.50 (0.74, 3.03)	
Latrine Use											
Yes	264	1	34	1	3	1	10	1	14	1	21
No	336	1.29 (0.85, 1.96)	39	3.53 (1.31, 9.57)*	9	1.68 (0.92, 3.07)	16	0.89 (0.51, 1.57)	13	1.02 (0.63, 1.64)	21
		1.37 (0.89, 2.10)		3.24 (1.18, 8.97)*		1.60 (0.86, 2.98)		0.92 (0.52, 1.64)		0.95 (0.57, 1.57)	
Hand Hygiene											
Clean	174	1	35	1	7	1	12	1	13	1	21
Unclean	426	1.25 (0.80, 1.93)	40	0.56 (0.21, 1.53)	4	1.38 (0.76, 2.53)	16	1.07 (0.58, 1.98)	14	1.13 (0.67, 1.88)	23
		1.62 (1.00, 2.62)*		0.56 (0.20, 1.61)		1.27 (0.67, 2.42)		1.16 (0.61, 2.20)		0.91 (0.52, 1.59)	
Finger Nail Hygiene											
Clean	186	1	31	1	2	1	13	1	9	1	24
Unclean	414	1.47 (0.94, 2.29)	39	3.67 (1.08, 12.50)*	8	1.08 (0.58, 2.02)	14	1.77 (0.90, 3.48)	16	0.77 (0.47, 1.27)	20
		1.67 (1.04, 2.67)*		3.84 (1.10, 13.41)*		1.03 (0.54, 1.97)		1.80 (0.91, 3.56)		0.73 (0.43, 1.24)	
Household Water Source											
Pipe	156	1	33	----	-	1	18	1	13	1	16
Hand pump	300	2.48 (1.26, 4.88)*	55	----	-	0.73 (0.30, 1.74)	14	1.56 (0.62, 3.95)	19	1.23 (0.52, 2.91)	19
		2.37 (1.14, 4.91)*		----	-	0.64 (0.25, 1.63)		1.75 (0.67, 4.60)		1.69 (0.68, 4.21)	
Wells & Streams	144	1.12 (0.50, 2.51)	35	----	-	0.49 (0.16, 1.54)	10	2.35 (0.85, 6.46)	26	2.13 (0.84, 5.42)	29
		1.01 (0.42, 2.39)		----	-	0.44 (0.13, 1.43)		2.70 (0.93, 7.88)		3.07 (1.10, 8.52)*	

§ Adjusted for age and sex. * Statistically significant at 0.05. *E. histolytica/dispar=Entamoeba histolytica* and /or *Entamoeba dispar*; *S. mansoni=Schistosoma mansoni*; *E. vermicularis=Enterobius vermicularis*; *H. Nana=Hymenolepis nana*. COR=odds ratio as computed by logistic regression model; AOR=adjusted odds ratio as computed by logistic regression model

Table 3.3 Associations of anaemia and malnutrition among school children, Ethiopia.

Independent Variables		Dependent Variables								
		Anaemia			Stunting			Low BMI-for-age		
	n	COR (95%CI)	%	AOR (95% CI)§	COR (95%CI)	%	AOR (95% CI)§	COR (95%CI)	%	AOR (95% CI)§
Age										
6-9	169	1	13		1	29		1	25	
10-15	431	1.37 (0.70, 2.67)	10	4.20 (1.46, 12.04)*	1.65 (1.11, 2.46)*	38	1.64 (0.84, 3.19)	2.06 (1.36, 3.12)*	40	1.27 (0.64, 2.52)
Gender										
Male	288	1	15		1	38		1	35	
Female	312	0.46 (0.26, 0.82)*	7	0.46 (0.26, 0.81)*	0.74 (0.53, 1.05)	31	0.73 (0.52, 1.02)	0.90 (0.64, 1.27)	33	0.89 (0.63, 1.25)
Family size										
≤ 7	372	1	9		1	29		1	37	
> 7	228	1.80 (0.74, 4.40)	15	1.86 (0.74, 4.66)	2.06 (1.16, 3.67)*	46	2.04 (1.14, 3.65)*	1.12 (0.63, 1.97)	40	1.06 (0.59, 1.89)
Latrine use										
Yes	264	1	8		1	30		1	30	
No	336	1.47 (0.72, 3.00)	11	1.25 (0.60, 2.59)	1.46 (0.96, 2.21)	39	1.42 (0.93, 2.16)	1.61 (1.07, 2.44)*	41	1.65 (1.08, 2.52)*
Hand hygiene										
Clean	174	1	6		1	33		1	33	
Unclean	426	4.05 (2.00, 8.22)*	20	3.20 (1.54, 6.65)*	1.40 (0.90, 2.18)	41	1.35 (0.85, 2.10)	1.59 (1.03, 2.47)*	44	1.76 (1.11, 2.80)*
Finger nail hygiene										
Clean	186	1	7		1	33		1	39	
Unclean	414	1.71 (0.76, 3.88)	11	1.51 (0.66, 3.47)	1.12 (0.72, 1.75)	36	1.12 (0.71, 1.75)	0.87 (0.56, 1.34)	35	0.90 (0.58, 1.39)
Household water source										
Pipe	156	1	9		1	31		1	42	
Hand pump	300	1.62 (0.55, 4.77)	14	1.93 (0.62, 6.00)	1.26 (0.63, 2.53)	36	1.29 (0.63, 2.66)	0.65 (0.33, 1.27)	32	0.47 (0.23, 0.98)*
Wells & streams	144	0.69 (0.16, 3.04)	7	0.83 (0.18, 3.86)	1.33 (0.59, 2.97)	37	1.39 (0.60, 3.22)	1.24 (0.57, 2.67)	47	0.87 (0.38, 1.98)
E. histolytica/dispar										
No	356	1	9		1	34		1	34	
Yes	227	1.78 (1.02, 3.12)*	14	1.95 (1.11, 3.44)*	1.12 (0.79, 1.59)	36	1.11 (0.78, 1.57)	0.97 (0.68, 1.38)	34	0.90 (0.63, 1.29)
Hookworm Infection†										
No	148	1	10		1	34		1	35	
Yes	31	3.77 (1.26, 11.32)*	28	4.60 (1.44, 14.65)*	2.23 (0.96, 5.19)	47	2.24 (0.96, 5.23)	0.62 (0.23, 1.65)	23	0.60 (0.22, 1.65)
S. mansoni Infection†										
No	296	1	10		1	34		1	35	
Yes	80	2.77 (0.92, 8.34)	14	2.60 (0.84, 8.11)	1.16 (0.64, 2.11)	35	0.79 (0.43, 1.46)	1.07 (0.59, 1.95)	34	0.85 (0.46, 1.58)

§ Adjusted for age and gender. * Statistically significant at 95% confidence interval (CI). †Statistical analysis done only for areas where the parasite was detected. COR=odds ratio as computed by logistic regression model; AOR=adjusted odds ratio as computed by logistic regression model. *E. histolytica/dispar=Entamoeba histolytica/Entamoeba dispar*; S. *mansoni=Schistosoma mansoni*

Discussion

Our findings revealed widespread prevalence of intestinal parasitosis and malnutrition among school children in Ethiopia. The prevalence of anaemia was 11%. Although there were slight differences between urban and rural schools, the overall magnitude of the problem was the same. Children with unclean hands were more likely to be anaemic and thin. Furthermore, open air defecation around the backyard contributed to the poor nutritional status of these children, which suggests the need to address environmental sanitation. Although, IPIs and individual hygiene and sanitation practices were not very clearly related, there were significant associations between not using latrines, unclean fingernails, and presence of hookworm.

The overall prevalence of intestinal parasitic infection in our data was higher than the 2008 national prevalence report for the country,[22] and studies conducted elsewhere.[23-25] The prevalence of soil-transmitted helminthiasis (10%) was in line with the 2008 WHO report (<20%) for the country,[26] while the prevalence of schistosomiasis was higher in our survey (13.4%) than the reported prevalence of <10%. These results might indicate a need for epidemiological updates on *S. mansoni*, as the prevalence within endemic communities may vary due to changes in local environment and other exposure-related factors.[27]

Anaemia prevalence in the study group was consistent with a recent prevalence report for the country[22] and for Africa, but was much lower than the global prevalence.[2] The significantly higher involvement of boys than girls in animal-keeping activities, hence keeping them away from the home environment, and frequent bathing and swimming in streams, thus higher rate of exposure to *S. mansoni* infection, might explain the higher anaemia prevalence among the gender group. In our data, the prevalence of stunting (low height-for-age) and thinness (BMI-for-age) were higher than other reports for the country,[22,28] but were within the range reported for Africa.[3]

The inconsistent pattern of associations observed between poor hygiene and sanitation practices and IPIs could be partially explained by the differences in transmission and epidemiological features between the different intestinal parasite species, leading to various confounding factors, since infections are related to a range of host and environmental aspects.[29] Another possible reason might be lack of statistical power in our data due to the observed low prevalence of some parasitic infections.

Associations between poor hygiene and sanitation practices and malnutrition are well documented,[30,31] although comparisons are difficult because of differences in study populations. However, a study on rural adolescent girls from the same region found associations between open-air defecation (lack of latrine) and thinness.[14]

In sharp contrast to other studies,[32-34] but in agreement with some studies,[35-37] we found no significant association between IPIs and malnutrition. The lack of association could be explained by the overall low worm loads[35] or could be due to a real absence of measurable differences. In this study, no differentiation was made between the invasive *E*. *histolytica* and the commensal *E*. *dispar* parasites. It is likely that most infections were due to *E*. *dispar*, and were therefore harmless and not associated with any disease. A study from Ethiopia reported high prevalence of the commensal *E*. *dispar* infection.[38]

The use of mobile phone technology improved demographic data collection and quality by avoiding paper records and the need for manual data entry. It enabled us to develop simple data collection forms, which could be completed on mobile phones without requiring mobile network connectivity. When the mobile network was available, the forms stored on the phones were uploaded to the server. The cost of the phones and maintaining battery power in remote areas usually make smooth data collection difficult. However, in this particular survey, limited electricity access was mitigated by using solar chargers and extra mobile batteries. The potential use and advantage offered by this technology for parasitological field surveys in resource-limited settings merits further investigation.

Interpretation of the results should be considered in respect of the study's limitations. For instance, we had enough statistical power to investigate associations for the most prevalent parasites, but lacked adequate power to draw meaningful conclusions for less prevalent parasites. We were also reluctant to do extensive multivariate analysis. This would have required a thorough consideration of all possible confounders for each variable of interest, resulting in an enormous amount of statistical testing, thereby increasing the risk of several Type-1 errors. The associations shown in this part of this study should, therefore, be regarded as exploratory, providing direction for in-depth investigations of patterns of associations between risk factors and parasites. Another limitation was the cross-sectional nature of the study, making any inference on causal relationship among variables impossible.

Our results emphasise the need for improvements in personal hygiene practices and hygiene-related education, in addition to increased access to clean water for hand washing and latrines to restrict contamination, which may ultimately improve the health of this age group. Randomised trials should be conducted to determine the causal relationship between personal hygiene, parasitic infection, and malnutrition.

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Chapter 4

Impact of hand-washing with soap and nail clipping on intestinal parasitic infections in school-aged children: a factorial randomized controlled trial

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Submitted

Abstract

Background

Intestinal parasitic infections are highly endemic among school-aged children in resource-limited settings. Preventive measures that are sustainable with available resources should be implemented to reduce the prevalence of infection. The aim of this study was to assess the impact of hand-washing with soap and nail clipping on the prevention of intestinal parasite re-infection.

Methods and findings

Three-hundred-sixty-seven parasite negative school-aged children (aged 6-15) were randomly assigned to receive both, one or the other, or neither of the interventions in a 2x2 factorial design. Assignment sequence was concealed. Following six months follow-up, stool samples were examined using direct, concentration, and Kato-Katz methods. Haemoglobin levels were determined using a HemoCue spectrometer. Primary study outcomes were prevalence of intestinal parasite re-infection and infection intensity. Secondary outcome was anaemia prevalence. Analysis was by intention-to-treat. Fourteen percent (95% CI: 9% to 19%) of the children in the hand-washing with soap intervention were re-infected versus 29% (95% CI: 22% to 36%) in the control (Adjusted odds ratio [AOR] 0.32, 95% CI: 0.17 to 0.62). Similarly, 17% (95% CI: 12% to 22%) of the children in the nail clipping group were re-infected versus 26% (95% CI: 20% to32%) in the control (AOR 0.51, 95% CI: 0.27 to 0.95). Significant reduction in arithmetic mean egg per gram (epg) of stool was observed among children in the hand-washing with soap and the nail clipping groups compared to the control group (p=0.005 and p=0.001, respectively). Likewise, following intervention, 13% (95% CI: 8% to 18%) of the children in the hand-washing group were anemic versus 23% (95% CI: 17% to 29%) in the control (AOR 0.39, 95% CI: 0.20 to 0.78). Prevalence of anaemia did not differ significantly between children in the nail clipping group and those in the control group (AOR 0.53, 95% CI: 0.27 to 1.04). The need for intensive follow-up and monitoring during this study makes that we observed the benefits of the interventions under rather ideal circumstances so possibly the effects are overestimated compared to usual conditions.

Conclusions

Hand-washing with soap at key times and weekly nail clipping decreased intestinal parasite re-infection rates and mean egg loads. Further, the hand-washing intervention significantly reduced the prevalence of anemia in the study population. The next essential step should be implementing pragmatic studies and developing more effective approaches to promote and implement hand-washing with soap and nail clipping at larger scales.

Introduction

IPIs are highly prevalent in the resource-limited regions of the world.[1] School-aged children are particularly susceptible to parasitic infections.[1,2] Both protozoan and helminthic infections correlate with unrecognized morbidities including growth deficits, malnutrition and poor school performance.[3] Furthermore, intestinal parasitic infections are reported to be substantially linked with anemia in children. Intestinal parasitic infections can decrease food and nutrient intake, cause intestinal blood losses and induce red blood cells destruction by the spleen.[4,5]

The current strategy to control intestinal worm infections is periodic treatment of people at risk.[6] However, providing anthelminthic drugs systematically is difficult,[7] and may increase potential drug resistance.[3] Furthermore, drug therapy alone only temporarily solves the problem, considering frequent re-infections in areas where IPIs are highly endemic.[2]

To reduce the dependency on a 'drug only' approach and to enhance sustainability, complementary measures should be implemented[8-10] which sustainable with available resources. Human hands are important vectors that carry disease-causing pathogens.[11] Hand-washing is one of the most important interventions proven to effectively reduce the incidence of infectious diseases.[12] Hand-washing, especially with soap, has been shown to be an effective diarrhoeal,[13,14] and respiratory[14,15] diseases preventive measure.

In contrast, very little information on the impact of hand-washing on IPIs and anaemia is available, and existing evidence for effective hand-washing is inconclusive.[16] In addition to unclean hands, dirty and untrimmed nails have been associated with high parasite infection in observational studies.[17,18] However, there is no evidence for a potential beneficial effect of nail clipping on parasitic infections.

Since the faecal-oral route is the main dissemination pathway for parasitic infections, it is reasonable to suggest that promotion of hand-washing with soap and hand nail clipping may reduce both prevalence and intensity of intestinal parasite re-infections. These interventions are simple and can be implemented in low-income settings. We conducted a factorial randomised controlled trial to assess the impact of hand-washing with soap and nail clipping on the prevalence of intestinal parasite re-infection, infection intensity, and prevalence of anemia among school-aged children in rural areas of Northern Ethiopia.

Methods

Ethical considerations

Ethical clearance was obtained from the institutional Ethical Review Board of the College of Health Sciences, Mekelle University, Ethiopia. All participants and/or their guardians gave a written informed consent/assent for participation. Information sheets were read to participants in local language (Tigrigna) with explanations about the proposed home based activities. Children who were diagnosed positive for intestinal parasitic infections at follow-up were treated with standard medication.[19]

Study design

A 2 x 2 factorial randomized trial was conducted in a rural area of Northern Ethiopia, to evaluate the impact of hand-washing with soap and nail clipping on intestinal parasite re-infection rates, infection intensity (as measured by arithmetic mean egg per gram of stool, epg), and anaemia prevalence among school-aged children after a 6-month study intervention period.

Setting and study population

A scattered rural community within the Demographic Health Surveillance (DHS) site was selected based on the high prevalence of intestinal parasitic infections among school children.[18] A total of 216 households with at least one school-aged child (aged 6-15) were randomly selected using the DHS household census as a sampling frame. In households with more than one school-aged child, two children were recruited randomly and were given the same intervention, resulting in a total number of 369 children in the study population.

Eligible children were aged 6-15, screened negative for IPIs either at baseline or after pre-trial anti-parasitic treatment, were planning to continue to reside in the same house for the study period, and had an informed consent signed by their parents or guardians. The exclusion criteria were a positive screen for IPIs after pre-trial treatment and suffering from severe physical and mental disabilities.

Randomisation

Eligible children were randomly assigned to receive both, one or the other, or no treatment interventions (Figure 4.1). One investigator who did not participate in recruiting the study participants randomly allocated the interventions using a computer-generated random numbers in pre-prepared sealed numbered envelopes. To facilitate blinding, the study was explained as an assessment of intestinal parasitosis among school-aged children, while the principal purpose of the trial was concealed.

Assignment sequence was concealed from researchers recruiting the study participants until after interventions were assigned. Laboratory personnel were blinded to group assignments and to the assessment outcomes. Participating children and their families were aware of the intervention they had received, but were blinded for the study hypothesis and the intervention(s) given to the other study groups.

Procedure

Following acquisition of a signed informed consent, a series of parasitological screening and treatment administration steps were carried out. Parasite-negative children were randomly assigned to interventions of hand-washing with soap, nail clipping, both, or to continue with existing habit and practices (Figure 4.1). All children were monitored for six months from June to November, 2012.

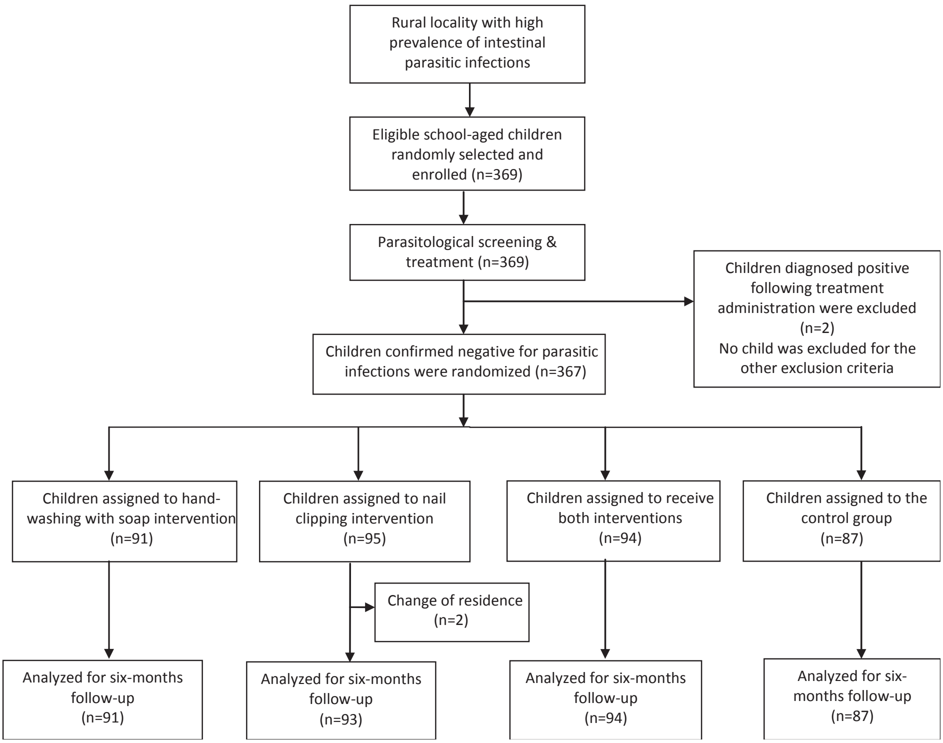

Figure 4.1 Trial profile, two-by-two factorial design.

Interventions

Hand-washing with soap

A total of eight fieldworkers were recruited to implement the intervention. They encouraged all individuals in the intervention household (who were old enough to understand) to wash their hands with water and soap before meals, after defecation, after playing on the ground, before preparing food, after cleaning an infant who had defecated, before feeding infants, and any other time when their hands were unclean. Initially, fieldworkers provided 2-4 bars of 120 g plain soap per household, depending on size. Soap was regularly replaced throughout the study period. Soaps were exclusively used for hand-washing as per the directives of the study.

Fieldworkers encouraged participants to wet their hands, lather them with soap, rub hands together for 45 seconds, and rinse the lather off with running water. Hands were dried with clean cloths prepared by the households. Children were instructed to hum a song for the time needed to rub their hands. Fieldworkers visited intervention households every week, for an average of 10-15 minutes each, to correct hand-washing techniques and promote regular hand-washing with soap at key times. Compliance was ascertained weekly by observing the size of the soap, the cleanliness of both hands, and asking participants to demonstrate the hand-washing procedures.

Nail clipping

New nail clippers were provided for groups assigned to the nail clipping intervention. Nail clipping was completed on a weekly basis by the fieldworkers. Nail clippers were kept by the fieldworkers tagged with code numbers for each child in the intervention groups for hygienic reasons, and clippers were replaced when necessary.

Control condition

Fieldworkers provided the control households with a regular monthly supply of sugar in an effort to preserve willingness to participate, but they gave no products that were expected to affect the hand-washing and nail clipping behaviour. They neither encouraged nor discouraged hand-washing or nail clipping in control households, and visited both control and intervention households with equal frequency. Control households were frequently checked for the presence of soap for hand-washing and nail clippers during the weekly visits to check for contamination of the study.

Outcome measures

The trial was stopped following six months follow-up. The main outcome measures of our study were reduction in the prevalence of intestinal parasite re-infection rates and egg counts among the intervention group. The secondary outcome measure was reduction in anaemia prevalence.

Sociodemographic data collection

Two separate structured questionnaires (child and household) were administered by the investigators in a local language during recruitment, which took place in April and May 2012, to generate information on the demographic, together with personal hygiene and sanitation behaviours.

Parasitological examination

Following intervention, fresh stool specimens of approximately 10 g were collected on the spot from all study subjects. Children were brought to the school where our lab was established, and provided with a clean labelled plastic screw-top container for sample collection, a plastics sheet to catch the stool in the toilet, and an applicator stick to transfer the sample. Stool specimens were analysed by well-trained blinded laboratory personnel using direct saline wet mount, formalin ethyl-acetate concentration technique[20], and the Kato-Katz technique (thick smear 41.7mg).[21] Duplicate slides were prepared for each stool specimen and for each of the techniques used. For the Kato preparations, averages were taken whenever differences in the counts were observed. Specimens were immediately processed and the Kato and wet mount preparations were analysed within 30 minutes to detect hookworm eggs and protozoan trophozoites (*Entamoeba histolytica/Entamoeba dispar* and *Giardia lamblia*, respectively). The remaining stool specimens were kept in 10% formalin and were examined using the concentration method within two hours after collection. Kato preparations were re-examined after 72 hours for the detection of helminth ova. A child was classified as re-infected if an infection was detected by any methods used.

The number of eggs per gram (epg) of stool was calculated using a conversion factor of 24 provided with the kit (Vestegraard Frandsen group, Denmark) by counting the number of eggs helminth parasites detected on each slide. Ten percent aliquots of stool smear samples were re-examined for quality control purposes.

Haemoglobin survey

At baseline and 6-months follow-up points, haemoglobin concentration was determined in finger prick blood using a HemoCue analyzer (HemoCue Hb 201z, Sweden).[22] Two microcuvette preparations were analyzed from a single blood specimen. The final measure was the mean of these two measurements. Technicians collecting and analyzing blood samples were trained for one day on machine operation before the actual data collection. Machines were checked on a daily basis using the reference microcuvettes as indicated by the manufacturer. Haemoglobin readings were adjusted for altitude, and anemia was defined for respective age and gender groups based on the World Health Organization cut-off values.[23]

Data analysis

The primary hypothesis of the study was that hand-washing with soap and nail clipping would significantly reduce the prevalence of intestinal parasite re-infection rates and faecal egg counts. Participants were analysed according to their randomised group allocation, consistent with intention-to-treat analysis.

We calculated a sample size of 216 households using the formula for comparison of proportion of successes,[24] which were evenly allocated into intervention and control groups. The required sample size was calculated based on the assumptions of a prevalence of 72%,[18] a minimum detectable difference of 20%, a power of 80%, a significance level of 0.05, and 20% drop-out. Sample size calculation was performed with an assumption of no interaction between the two factors. Separate sample size calculations were carried out based on target effect sizes for each of the intervention and the larger sample size was taken as the trial sample size to enable the trial to be powered to detect the main effects of each intervention.

Statistical analysis was conducted using Stata 13.1. Our analysis first focused on the main effects of the interventions, as is customary for studies with factorial designs, but the effects were also analysed for the four groups separately. Following the main effect analysis, effect modification was investigated by adding an interaction term to the regression models. Multilevel logistic regression models were used to investigate the efficacy of the interventions in reducing intestinal parasite re-infection rates and anaemia prevalence by odds ratios (ORs) and 95% confidence interval (CI). The main effects analysis included both factors (hand-washing with soap and nail clipping) in the same additive model. The main effects were adjusted for child gender, age, drinking water source, latrine use, baseline hand-washing with soap and nail hygiene, parasite at baseline and the other factor in the additive model. For the secondary outcome, the effect was adjusted for gender, age, water source, latrine use, anaemia at baseline, hand-washing at baseline, nail hygiene at baseline and the other factor in the additive model. Difference in arithmetic mean for the main egg counts was analysed using multilevel linear regression models. The statistical significance was set at $p<0.05$.

Results

Of the 369 school-aged children selected for the study, two were excluded before randomization and another two children were lost-to-follow up due to change in residential area (Figure 4.1). Boys accounted for 41% (n=150) of the study participants and the mean age was 10 years (SD=2.6). At baseline, children in the four intervention groups were similar in terms of age and sex distribution, and in their personal hygiene and sanitation practices (Table 4.1). Households had a mean of 5.9 members (SD=2.0). During the study, households assigned to hand-washing intervention received an average of 1.5 bars of 120 g of soap per week; thus, about 4.4 g of soap was used per person in the intervention group per day. Throughout the course of the follow-up no soap for hand-washing and nail clippers were observed in the control households.

Table 4.1 Base line demographic and hygiene characteristics by intervention group (n=367).

		Intervention and control groups			
Baseline characteristics	Overall (n=367) n (%)	Hand-washing with soap (n=91) n (%)	Finger nail clipping (n=95) n (%)	Hand-washing with soap and nail clipping (n=94) n (%)	Control (n=87) n (%)
Gender					
Male	150 (41)	38 (41)	39 (41)	38 (40)	35 (40)
Female	217 (59)	53 (59)	56 (59)	56 (60)	52 (60)
Age					
6-9	161 (44)	40 (44)	42 (44)	41 (44)	38 (44)
10-15	206 (56)	51 (56)	53 (56)	53 (56)	49 (56)
Hand-washing with soap					
Yes	46 (13)	14 (15)	13(14)	9 (10)	10 (11)
No	321 (87)	77 (85)	82 (86)	85 (90)	77 (89)
Hand-washing before meal‡					
Yes	350 (95)	86 (95)	92 (97)	89 (95)	83 (95)
No	17 (5)	5 (5)	3 (3)	5 (5)	4 (5)
Hand-washing after defecation†					
Yes	50 (14)	11 (12)	13 (14)	14 (15)	12 (14)
No	317 (86)	80 (88)	82 (86)	80 (85)	75 (86)
Nail hygiene					
Trimmed	90 (25)	25 (27)	22 (23)	25 (27)	18 (21)
Untrimmed	277 (75)	66 (73)	73 (77)	69 (73)	69 (79)
Drinking water source					
Pipe	83 (23)	16 (18)	15 (16)	22 (23)	32 (37)
Hand pump	239 (65)	64 (70)	64 (67)	60 (64)	48 (55)
Wells and streams	45 (12)	11 (12)	16 (17)	12 (13)	7 (8)
Latrine use					
Yes	140 (38)	39 (43)	35 (37)	31 (33)	35 (40)
No	227 (62)	52 (57)	60 (63)	63 (67)	52 (60)

‡ using water only; † both water only and water and soap

Main outcome

Table 4.2 describes the descriptive and multivariate regression analysis results for the main outcome. The interaction between the interventions was investigated as secondary analysis and was found to be not significant (p=0.069). After six months follow-up, 14% (95% CI: 9% to 19%) of the children received hand-washing with soap intervention were re-infected compared to 29% (95% CI: 22% to 36%) of the children in the control group (AOR 0.32, 95% CI: 0.17 to 0.62). Similarly, 17% (95% CI: 12% to 22%) of the children in the nail clipping group were re-infected, compared to 26% (95% CI: 20% to 32%) in the control group (AOR 0.51, 95% CI: 0.27 to 0.95).

Table 4.2 Intestinal parasite re-infection rates at six months follow-up (n=367).

		Finger nail clipping		
		Yes	No	Margin
Hand-washing with soap	Yes	14% (13 out of 94 children) OR 0.24 (CI 0.10 to 0.55) AOR 0.19 (CI 0.08 to 0.48)	14% (13 out of 91 children) OR 0.25 (CI 0.11 to 0.57) AOR 0.19 (CI 0.08 to 0.47)	14% (26 out of 185 children) OR 0.36 (CI 0.20 to 0.66) AOR 0.32 (CI 0.17 to 0.62)
	No	21% (20 out of 95 children) OR 0.42 (CI 0.20 to 0.88) AOR 0.32 (CI 0.14 to 0.73)	38% (33 out of 87 children) OR 1 (Ref)	29 % (53 out of 182 children) OR 1 (Ref)
	Margin	17% (33 out of 189 children) OR 0.54 (CI 0.33 to 1.03) AOR 0.51 (CI 0.27 to 0.95)	26% (46 out of 178 children) OR 1 (Ref)	

ORs (crude odds ratios) and AORs (Adjusted odds ratios) are comparisons for the intervention with the control. AORs are adjusted for gender, age, drinking water source, latrine use, parasite at baseline, hand-washing with soap at baseline, nail clipping at baseline and the other factor in the additive model. Interaction between the interventions in the adjusted model was not significant, p=0.069.

The arithmetic mean number of eggs for the children in the hand-washing group was 4.1 (SD=16.3) versus 11.8 (SD=35.1) in the control group. This difference was statistically significant with an adjusted arithmetic mean difference between the groups of 7.86 and a p-value of 0.005. Similarly, a significant difference in the arithmetic mean egg counts was observed in children in the nail clipping group 3.4 (SD=15.0) and the control group 12.6 (SD=35.7), with an adjusted arithmetic mean difference of 9.10 and a p-value of 0.001. Also in this case the interaction between both interventions was not significant (p=0.069).

Secondary outcome

Descriptive and multivariate logistic regression analysis results for the secondary outcome are described in Table 4.3. The interaction between the interventions was found to be not significant (p=0.814). At the end of the trial, 13% (95% CI: 8% to 18%) of the children in the hand-washing intervention were anaemic compared to 23% (95% CI:

17% to 29%) in the control group (AOR 0.39, 95% CI: 0.20 to 0.78). Similarly, 14% (95% CI: 10% to 18%) children in the nail clipping intervention were anemic compared to 21% (95% CI: 17% to 25%) in the control group, however, the observed difference was not statistically significant (AOR 0.53, 95% CI: 0.27 to 1.04).

Table 4.3 Anaemia prevalence at six months follow-up (n=367).

		Finger nail clipping		
		Yes	No	Margin
Hand-washing with soap	Yes	12% (11 out of 94 children) OR 0.22 (CI 0.08 to 0.58) AOR 0.21 (CI 0.08 to 0.58)	14% (13 out of 91 children) OR 0.38 (CI 0.16 to 0.92) AOR 0.37 (CI 0.15 to 0.91)	13% (24 out of 185 children) OR 0.40 (CI 0.21 to 0.78) AOR 0.39 (CI 0.20 to 0.78)
	No	17% (16 out of 95 children) OR 0.51 (CI 0.22 to 1.20) AOR 0.49 (CI 0.21 to 1.19)	29% (25 out of 87 children) OR 1 (Ref)	23 % (41 out of 182 children) OR 1 (Ref)
	Margin	14% (27 out of 189 children) OR 0.59 (CI 0.33 to 1.03) AOR 0.53 (CI 0.27 to 1.04)	21% (38 out of 178 children) OR 1 (Ref)	

ORs (crude odds ratios) and AORs (Adjusted odds ratios) are comparisons for the intervention with the control. AORs are adjusted for gender, age, drinking water source, latrine use, baseline hand-washing with soap, nail hygiene at baseline and the other factor in the additive model. The interaction between the interventions in the adjusted model was not significant, p=0.814.

Discussion

The purpose of this trial was to evaluate the impact of two simple public health interventions (hand-washing with soap and finger nail clipping) on the risk of intestinal parasite re-infection, infection load, and anaemia among school-aged children.

Our six-month hand-washing with soap and weekly nail clipping interventions demonstrated a significant reduction in intestinal parasite re-infection rates and the size of worm burden acquired after a successful treatment. Children who received hand-washing with soap at critical times were 68% less likely to be re-infected by intestinal parasitic infections than children left to continue with existing habit and practice. Similarly, children who cut their nails on a weekly basis were 49% less likely to be re-infected by intestinal parasitic infections than the control group. Furthermore, children who received hand-washing with soap were 61% less likely to be anaemic than children in the control group.

Several observational studies indicated the impact of hand-washing on the prevention of IPIs.[25-28] A case-control study from Vietnam demonstrated a significant reduction in the risk of *E. histolytica* infection among individuals who frequently washed their hands with soap.[27] A longitudinal cohort study by Monse and his colleagues,[28] also demonstrated decreased re-infection rates with soil-transmitted helminthes (STH) among school children who washed their hands with soap. However, most studies did not take into account whether soap was effectively used. They used self-reported hand-washing behaviour as their exposure measure - a major methodological weakness that was addressed in the present study. Furthermore, to our knowledge, there have been no randomized control trials conducted to address the causal impact of hand-washing with soap and nail clipping on IPIs, worm infection intensity and anaemia.

Significant reductions in the prevalence of anemia among children were reported from an interventional study that integrated hand-washing and dietary modification interventions.[29] The confounding effects of the deworming and dietary modification among the intervention group may result in difficulty in identifying the component responsible for the reported improvements. Further, the studies were not designed to allow causal inference.

In addition to the immediate benefits of improving the health of children, proper hand-washing with soap and weekly trimming of the fingernails can lead to a reduction in the distribution of infective parasites from faeces that result in the contamination of the environment and hence, can reduce infection transmission in the community.[3,30] In helmenthiasis, the intensity of infection is of epidemiological importance as it is the central determinant of transmission dynamics and morbidity.[1,31,32] The observed reduction of the overall worm burden following enhanced hand hygiene interventions would probably contribute to the overall reduction of transmission and sustain gains in the long term. To confirm this hypothesis, pragmatic trials involving a larger community than those who received the intervention is needed. Interventions involving hand-washing are also documented to have a lasting pedagogical effect by decreasing infectious illness and hence decreasing school absenteeism.[33]

Strength and weaknesses

Our study demonstrated causal relationships between hand hygiene and infection, and anaemia among school-aged children. Although our data showed that hand-washing and nail clipping were efficacious, our trial included intense follow-up and monitoring involving high human resource investment. The long-established habitual and culturally embedded practices on personal hygiene and sanitation among children and households might require additional methods that would enhance behavioural changes and hence would make large scale implementations of such interventions more expensive. Furthermore, as any other efficacy study, intervention benefits were assessed under specific conditions and hence might limit the generalizability of the results and overestimate the intervention effects when implemented under usual circumstances.

Since labour is relatively cheap in Ethiopia and other low income countries, national campaigns might be organised to implement hand-washing with soap at key times and weekly nail clipping through house to house education. Additionally, hand-washing and nail clipping interventions could be integrated into existing community health programmes (for example, the Health Extension Program, Demographic Surveillance Sites, and Health Development Army network) in the country that reach inaccessible impoverished populations through house-to-house visits as their outreach activities. The next essential step should focus on implementing pragmatic studies that investigate the performance of the interventions under circumstances that more closely approach the real and usual conditions and developing additional more effective approaches to promote hand-washing with soap and nail clipping at larger scale.

Conclusion

Our data showed that regular hand-washing with soap and nail clipping are efficacious in preventing intestinal parasitic re-infections and reducing infection intensity, and thereby deliver health benefits to school-aged children at risk. Proper hand-washing and weekly nail clipping may be considered for widespread implementation as a public health measure across societies of resource-limited regions to reduce transmission of IPIs.

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Chapter 5

Associations between intestinal parasitic infections, anaemia, and diarrhoea among school-aged children, and the impact of hand-washing and nail clipping

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Submitted

Abstract

Background

In marginalised settings, under-nutrition and illnesses due to infectious agents create a vicious circle. Children are especially vulnerable to the deleterious effects of infection, anaemia and diarrhoea. Our aims have been to assess the pattern of associations between intestinal parasitic infections (IPIs), anaemia, and diarrhoea among school-aged children; to asses baseline predictors for IPIs, anaemia and thinness; and to explore the impact of hand-washing and nail clipping across children with different baseline demographic and disease characteristics.

Methods

The study was part of a factorial randomised controlled trial where the impact of hand hygiene interventions on intestinal parasite re-infection was assessed with a follow-up period of six months. Associations between IPIs, anaemia and diarrhoea, and baseline predictors for infection and anaemia were analysed using binary and multiple logistic regression models with odds ratios (OR) and 95% confidence intervals (CI). Furthermore, impact of hand-washing with soap and nail clipping on the primary end point (intestinal parasite re-infection rates) was analysed across children with different backgrounds, using logistic regression models. Possible moderating effect of each baseline variable on the effect of the interventions was identified by adding interaction terms to the regression models.

Results

Children with IPIs had a much higher chance of also being anaemic (OR 2.09, 95% CI: 1.15 to 3.80), having diarrhoea (OR 2.83, 95% CI: 1.57 to 5.09), and vice versa. Anaemia and diarrhoea were very strongly related (OR 9.62, 95% CI: 5.18 to 17.85), meaning that children with diarrhoea had a very high chance of also having anaemia, and vice versa. Our analysis on baseline predictors for IPIs, anaemia, and thinness revealed no relevant trend of associations. Anaemia seemed more common in younger children (6-9 years) (OR 1.82, 95% CI: 1.13 to 2.93). The presence of pre-intervention IPIs was statistically associated with post-intervention parasites (OR 1.98, 95% CI: 1.19 to 3.28). Similarly, the presence of pre-intervention anaemia was also associated with post-intervention parasites (OR 2.08, 95% CI: 1.23 to 3.51). Overall, hand-washing with soap at key times and weekly nail clipping were efficacious in preventing intestinal parasite re-infection among children despite the differences in their baseline demographic characteristics.

Conclusions

Intestinal parasitosis, anaemia and diarrhoea were independently associated among school-aged children. Hand-washing with soap at key times and weekly nail clipping were efficacious in reducing intestinal parasite re-infection rates across the children regardless of their baseline demographic differences.

Introduction

In resource-poor settings, under-nutrition and illnesses due to infectious diseases are highly prevalent and closely interlinked. Nutritional deficiencies predispose people to infection, and infections lead to nutritional deficiencies which further reduce resistance to new infections.[1-3] Children are especially susceptible to the deleterious effects of under-nutrition[4] and infections[5] in developing settings.

In Ethiopia, similar to many developing countries, IPIs[6,7] and anaemia[6,8] are common among school-aged children. This suggests that school-aged children in Ethiopia may be vulnerable to the cyclical ill-effects of anaemia and illnesses due to parasitic infections. However, there is insufficient evidence regarding the associations of IPIs, anaemia and diarrhoea among these population groups in the country.

Remarkable heterogeneity is documented in the distribution patterns of IPIs.[9] Several demographic[10], socioeconomic[11], and environmental[12] factors influence the distribution patterns of IPIs within a community. These factors can create different distributions of susceptibility to re-infection by IPIs among a population, and hence, different groups of children may be at different risks of parasitic re-infection.

A randomised controlled trial (described in Chapter 4) revealed a significant impact of regular hand-washing with soap on the prevention of parasitic re-infection and anaemia among children. It is unknown if certain characteristics of the children also determined the effects of hand-washing. One could imagine, for example, that in areas where parasites are distributed by polluted water, that hand-washing would not be effective in preventing parasitic infections. We set out to explore if the effect of hand-washing and nail clipping interventions in Chapter 4 was similar across the study population despite their different characteristics.

Description of the trial

A total of 216 households with at least one school-aged child (aged 6-15 years) were randomly selected from the Demographic Health Surveillance (DHS) site of the College of Health Sciences of Mekelle University. When the selected household had more than one child in the age group, two children were recruited randomly and given the same intervention. Overall, the study population comprised 369 apparently healthy school-aged children.

Prior to group allocation and implementation of the interventions, children were screened for intestinal parasitosis. Parasitological analysis using direct, formalin ethyl-acetate concentration technique[13] and the Kato-Katz technique[14] was carried out. If children were screened positive, treatment was administered.[15] Children screened negative for intestinal parasitosis, following treatment administration, were recruited and randomly assigned to the different intervention and control groups.

An official not involved in recruiting study participants prepared sealed and numbered randomisation envelopes at the study centre using computer generated random numbers, and randomly assigned the study participants to the treatment and control groups. Assignment sequence was concealed until interventions were assigned. Field workers analysing the stool specimens, taking anthropometric and haemoglobin measurements, and registering diarrhoeal incidence were blinded to group assignments and to the assessment outcomes.

Interventions

Hand-washing with soap

The detailed procedures for the trial have been described in Chapter 4. Briefly, plain soaps were provided and study households were encouraged to wash their hands at key times. Fieldworkers visited intervention households at least once a week to promote regular hand-washing habits. Each enrolled participant was instructed on the procedures of hand-washing with soap and the time needed to rub hands with the lather (45 seconds).

Finger nail clipping

New nail clippers were provided for each child and nail clipping was performed by the field workers on weekly basis. Nail clippers were kept by the field workers and tagged with code numbers for each child. Nail clippers were replaced when necessary throughout the study period.

Control condition

Children in the control group were neither encouraged nor discouraged to wash their hands or cut their finger nails. Control children were visited with equal frequency.

Data collection

Baseline factors

At the beginning of the trial, structured questionnaires (both for child and household) were administered by the investigators in a local language to generate relevant baseline data. Basic sociodemographic and disease-related variables were selected to assess for known risk factors for intestinal parasitosis.[16-20] Baseline variables covered two domains: (1) sociodemographic, which included age, gender, drinking water source, latrine use, house ownership, maternal age, education, and family size; and

(2) data on history of parasitic infections, anaemia and Body-Mass-Index (BMI)-for-age (thinness).

Parasitological analysis

Following six months follow-up, fresh stool specimens were collected from the study subjects. Stool specimens were analysed using direct saline wet mount, formalin ethyl-acetate concentration technique[13] and the Kato-Katz technique.[14] A child was classified as re-infected if an infection was detected by any methods used. Sub-samples of stool smears, comprising 10% of the total, were re-examined for quality control purposes.

Anaemia

Baseline and six-month haemoglobin levels were determined by finger prick blood using a HemoCue analyser (HemoCue Hb 201z, Sweden).[21] Haemoglobin readings were adjusted for altitude, and anaemia was defined for respective age and gender groups based on WHO cut-off values.[22]

Diarrhoea incidence

Data on self-reported diarrhoeal episodes were collected using a separate questionnaire on a weekly basis during the study period. Diarrhoea was defined as the passage of three or more loose or liquid stools per day.[23] Presence of diarrhoea in the week was determined through information gathered from either the parents or the child.

Anthropometry

Anthropometric measurements were taken at the start and the end of the follow-up in duplicate, by two independent, trained and blinded data collectors. The average of the two measurements was recorded. Portable weight scales and locally made stadiometers with a sliding headpiece were used to measure weight (to the nearest 0.1 kg) and height (to the nearest 0.1 cm), respectively. Each child was weighed with minimal clothing and barefooted. The weighing scales were calibrated using standard calibration weights of 5 kg iron bars. Height measurements were taken with children faced forwards, barefooted with feet flat and together on the centre of the base with their heels and back against the rod. Anthropometric measurements were converted into BMI-for-age Z scores using WHO AnthroPlus software, version 1.0.4 (WHO Anthro 2007, WHO, Geneva, Switzerland). Children below -2Z scores for BMI-for-age were classified as thin.

Statistical analysis

Statistical analysis was completed using SPSS for Windows version 16.0 (Chicago, USA). Associations between post-intervention IPIs, anaemia, and diarrhoea; and baseline predictors for infection and anaemia were analysed using binary and multivariate logistic regression models by odds ratios (OR) and 95% confidence intervals (CI). The impact of hand-washing with soap and nail clipping on the primary end point (intestinal parasite re-infection rates) was analysed across children with different backgrounds using logistic regression models. Stratifications included baseline demographics (child age, gender, latrine use, maternal education, maternal age, family size, family drinking water source, and living house ownership) and pre-existing disease characteristics (IPIs, anaemia, and thinness). Possible moderating effects of each baseline variable on the effect of intervention were identified by adding interaction terms to the regression model.[24] For all analyses, alpha was set at 0.05 levels for statistical significance.

Ethical considerations

The study protocol and informed consent procedure for the initial randomised controlled trial was approved by the Institutional Review Board of the College of Health Sciences, Mekelle University, Ethiopia. Informed consent was obtained from each child's parents and/or guardians. Children diagnosed positive for IPIs at follow-up were treated with standard medication,[15] and children with anaemia and/or diarrhoea were sent to the health facilities for further medical attention.

Results

Baseline characteristics and the trial profile of the original project have been described previously (Chapter 4). Briefly, 365 (99%) children were analysed for six-month follow-up. Boys comprised 41% (n=150) of the study participants and mean age was 10 (SD=2.6) years. Following 6 months follow-up, 21 % (95% CI: 17% to 25%) of the children were re-infected with intestinal parasites, 18% (95% CI: 14% to 22%) of the children were anaemic and 17% (95% CI: 13% to 21%) had diarrhoea.

Associations between intestinal parasitosis, diarrhoea, and anaemia

Table 5.1 describes the multivariate logistic regression analysis results of the associations between intestinal parasitosis, anaemia, and diarrhoea. Effects were adjusted for each intervention. Current intestinal parasitosis, a history of diarrhoea in the previous week, and current anaemia were independently associated. IPIs were significantly associated with anaemia (OR 2.09, 95% CI: 1.15 to 3.80) and diarrhoea (OR 2.83, 95% CI: 1.57 to 5.09), and vice versa. Anaemia and diarrhoea were also strongly related (OR 9.62, 95% CI: 5.18 to 17.85), meaning that children with diarrhoea had a very high chance of also having anaemia, and vice versa.

Table 5.1 Associations between intestinal parasitic infections, anaemia and diarrhoea among school-aged children, Ethiopia (n=365).

	Post-intervention out-come variables		
	IPI	Anaemia	Diarrhoea
Post-intervention out-come variables	AOR (CI)	AOR (CI)	AOR (CI)
IPI	------	2.09 (1.15 to 3.80)*	2.83 (1.57 to 5.09)*
Anaemia	2.09 (1.15 to 3.80)*	------	9.62 (5.18 to 17.85)*
Diarrhoea	2.83 (1.57 to 5.09)*	9.62 (5.18 to 17.85)*	------

* statistically significant at 0.05. CI=95% confidence interval. AOR=adjusted odds ratio as computed by the logistic regression model. IPI=intestinal parasitic infection.

Predictors of IPIs, anaemia and thinness

Multivariate logistic regression analysis results for baseline predictors are shown in Table 5.2. Some variables were associated with having IPIs in the direction that would be expected, such as a positive association of having parasites when the household did not have a latrine, or when the mother was older or illiterate. However, most associations were not strong enough to reach statistical significance. The presence of pre-intervention IPIs and anaemia was significantly associated with post-intervention parasitic infections (OR 1.98, 95% CI: 1.19 to 3.28, and OR 2.08, 95% CI: 1.23 to 3.51, respectively). Anaemia seemed more common in younger children (6-9 years) (OR 1.82, 95% CI: 1.13, 2.93), but other variables did not show a clear pattern. We found no clear associations for low BMI.

Impact of hand-washing and nail clipping among children with different baseline demographic and disease background

As reported in Chapter 4, both hand-washing with soap (AOR 0.32, 95% CI: 0.20 to 0.62, p=0.001) and weekly finger nail clipping (AOR 0.51, 95% CI: 0.27 to 0.95, p=0.035) interventions had a significant impact in reducing the prevalence of intestinal parasite re-infection among the study participants.

In this study, we explored if these impacts were similar across children with different demographic and disease backgrounds. Overall, interventions seem equally efficacious among children regardless of age, gender, drinking water source, latrine use, mother's age, mother's education, family size, house ownership, and history of intestinal parasitosis, anaemia and thinness at baseline (Table 5.3). The impact of hand-washing was similar for the whole group and for children who had IPIs at baseline, but the effect significantly increased in children who were parasite-free at baseline (OR 0.48 vs. OR 0.31, p=0.048). The effects of hand-washing and nail clipping were higher for children whose drinking water sources were wells and streams compared to those who used pipeline and boreholes, but the effects were not statistically significant (OR 0.44 vs. OR 0.08, p=0.134 and OR 0.68 vs. OR 0.09, p=0.053; respectively).

Table 5.2 Predictors of pre-intervention and post-intervention intestinal parasitic infections, anaemia and thinness: results from the multivariate regression analysis.

Baseline characteristics		Intestinal Parasitic Infections		Anaemia		Low BMI-for-age	
		Pre-Intervention AOR (95% CI)‡	Post-Intervention AOR (95% CI)†	Pre-Intervention AOR (95% CI)‡	Post-Intervention AOR (95% CI)†	Pre-Intervention AOR (95% CI)‡	Post-Intervention AOR (95% CI)†
Gender	Female	1	1	1	1	1	1
	Male	0.70 (0.45, 1.09)	0.95 (0.44, 2.03)	1.58 (0.99, 2.53)	0.66 (0.31, 1.42)	1.16 (0.75, 1.79)	1.73 (0.89, 3.37)
Age	10-15 years	1	1	1	1	1	1
	6-9 years	1.26 (0.81, 2.00)	0.95 (0.43, 2.12)	1.82 (1.13, 2.93)*	1.70 (0.83, 3.50)	0.71 (0.46, 1.10)	0.82 (0.42, 1.61)
Drinking water source							
Water wells and streams		1	1	1	1	1	1
Pipeline and/or bore holes		1.14 (0.59, 2.20)	0.82 (0.24, 2.85)	0.93 (0.45, 1.89)	0.92 (0.31, 2.72)	0.95 (0.50, 1.83)	0.43 (0.17, 1.12)
Latrine use	No	1	1	1	1	1	1
	Yes	0.83 (0.53, 1.29)	0.62 (0.28, 1.40)	0.96 (0.58, 1.51)	1.37 (0.67, 2.81)	1.35 (0.87, 2.09)	1.12 (0.56, 2.22)
Mother's age	>35 years	1	1	1	1	1	1
	≤35 years	0.50 (0.32, 0.79)*	0.91 (0.41, 2.02)	1.37 (0.84, 2.23)	1.23 (0.58, 2.57)	0.77 (0.49, 1.19)	1.49 (0.73, 3.05)
Mother's education	Illiterate	1	1	1	1	1	1
	Literate	0.83 (0.53, 1.29)	0.48 (0.22, 1.07)	1.04 (0.64, 1.70)	1.48 (0.72, 3.04)	0.88 (0.56, 1.38)	1.02 (0.51, 2.07)
Family size	>6 members	1	1	1	1	1	1
	≤6 members	0.87 (0.55, 1.36)	2.40 (1.04, 5.54)*	0.68 (0.41, 1.11)	0.86 (0.40, 1.83)	0.70 (0.45, 1.10)	1.05 (0.52, 2.13)
House ownership	No	1	1	1	1	1	1
	Yes	0.75 (0.41, 1.37)	0.62 (0.23, 1.65)	1.29 (0.65, 2.55)	1.08 (0.41, 2.84)	0.69 (0.38, 1.24)	0.53 (0.22, 1.28)
Pre-intervention IPIs	No	1	1	1	1	1	1
	Yes	----	1.98 (1.19, 3.28)*	0.87 (0.54, 1.38)	1.15 (0.66, 1.98)	0.80 (0.55, 1.28)	0.86 (0.55, 1.34)
Pre-intervention anaemia status							
	Non- anaemic	1	1	1	1	1	1
	Anaemic	0.87 (0.54, 1.38)	2.08 (1.23, 3.51)*	----	1.17 (0.65, 2.11)	0.72 (0.45, 1.14)	0.78 (0.48, 1.29)
Pre-intervention low BMI-for-age							
	Normal	1	1	1	1	1	1
	Thinness	0.84 (0.55, 1.28)	1.30 (0.79, 2.15)	0.72 (0.45, 1.14)	1.33 (0.77, 2.29)	----	2.53 (1.62, 3.96)*

* Statistically significant at $p=0.05$. ‡ adjusted for each baseline variable. † adjusted for hand-washing, nail clipping and each baseline variable. AOR=adjusted odds ratio as computed by the logistic regression model. CI=95% confidence interval. IPIs=intestinal parasitic infections.

Table 5.3 Impact of hand-washing and nail clipping on parasite re-infection rates across children with different baseline demographic and disease characteristics (n=365).

	Hand-washing vs. control		Nail clipping vs. control	
	Subgroup N (%) AOR (CI)† p-value	Subgroup N (%) AOR (CI)†	Subgroup N (%) AOR (CI)† p-value	Subgroup N (%) AOR (CI)†
Gender	Male 150 (41%) 0.41 (0.17, 0.97) 0.923	Female 215 (49%) 0.39 (0.20, 0.75)	Male 150 (41%) 0.34 (0.14, 0.82) 0.131	Female 215 (49%) 0.79 (0.41, 1.51)
Age	6-9 years 208 (57%) 0.30 (0.13, 0.66) 0.338	10-15 years 157 (43%) 0.50 (0.25, 1.01)	6-9 years 208 (57%) 0.50 (0.23, 1.09) 0.604	10-15 years 157 (43%) 0.66 (0.33, 1.31)
Water source	Pipeline & borehole 321 (88%) 0.44 (0.25, 0.76) 0.134	Wells & streams 44 (12%) 0.08 (0.01, 0.68)	Pipeline & borehole 321 (88%) 0.68 (0.39, 1.17) 0.053	Wells & streams 44 (12%) 0.09 (0.13, 0.64)
Latrine use	Yes 139 (38%) 0.33 (0.13, 0.81) 0.744	No 226 (62%) 0.44 (0.23, 0.84)	Yes 139 (38%) 0.48 (0.20, 1.19) 0.791	No 226 (62%) 0.61 (0.32, 1.16)
Mother education	Literate 161 (44%) 0.58 (0.26, 1.30) 0.198	Illiterate 204 (56%) 0.28 (0.14, 0.58)	Literate 161 (44%) 0.83 (0.37, 1.85) 0.207	Illiterate 204 (56%) 0.42 (0.21, 0.84)
Maternal age	≤ 35 193 (53%) 0.22 (0.09, 0.51) 0.085	> 35 172 (47%) 0.58 (0.28, 1.22)	≤ 35 193 (53%) 0.58 (0.21, 1.58) 0.787	> 35 172 (47%) 0.71 (0.25, 2.02)
Family size	≤ 6 members 208 (57%) 0.27 (0.13, 0.54) 0.082	> 6 members 157(43%) 0.69 (0.31, 1.55)	≤ 6 members 208 (57%) 0.60 (0.30, 1.17) 0.814	> 6 members 157(43%) 0.53 (0.23, 1.20)
House ownership	Yes 307(84%) 0.45 (0.26, 0.79) 0.661	No 58 (16%) 0.17 (0.03, 0.88)	Yes 307(84%) 0.71 (0.40, 1.24) 0.386	No 58 (16%) 0.19 (0.05, 0.77)
Baseline parasitic infection status	Yes 146 (40%) 0.48 (0.23, 1.01) 0.048*	No 219 (60%) 0.31 (0.14, 0.67)	Yes 146 (40%) 0.44 (0.20, 0.97) 0.696	No 219 (60%) 0.69 (0.34, 1.43)
Baseline anaemia status	Yes 106 (29%) 0.59 (0.25, 1.39) 0.577	No 259 (71%) 0.32 (0.16, 0.64)	Yes 106 (29%) 0.54 (0.23, 1.26) 0.366	No 259 (71%) 0.60 (0.31, 1.15)
Baseline BMI-for-age	Thin 150 (41%) 0.33 0.15, 0.75) 0.577	Normal 215 (59%) 0.45 (0.23, 0.91)	Thin 150 (41%) 0.44 (0.20, 0.97) 0.366	Normal 215 (59%) 0.71 (0.36, 1.42)

†Adjusted for each intervention for the other one. CI=95% Confidence Interval. * Statistically significant at 0.05. AOR=Adjusted Odds Ratio.

Discussion

Findings of the present study demonstrate a clear relationship between IPIs, anaemia and diarrhoea among children. Children with IPIs had a much higher chance of also being anaemic and having diarrhoea. Anaemia and diarrhoea were also very strongly related, as children with diarrhoea had a very high chance of also having anaemia, and vice versa. Overall, our analysis for baseline predictors did not show a clear pattern. Most associations were not strong enough to reach statistical significance. The effects of hand-washing with soap and nail clipping on reducing intestinal parasite re-infection were independent of demographic differences among the children.

Associations observed between anaemia and IPIs in our data concord with other studies that showed IPIs to be substantially linked with anaemia in children.[25-28] IPIs can decrease food and nutrient intake, cause intestinal blood losses, induce red blood cell destruction by the spleen, and induce autoimmune reactions leading to chronic inflammation.[27-28] These effects may have accounted for the considerable proportion of anaemia observed among the children infected with intestinal parasites.

Although in most instances IPIs are asymptomatic, they may also cause diarrhoea.[29] IPIs can induce diarrhoea by increasing small intestine motility while reducing its digestive and absorptive capacities.[30] Our finding that diarrhoea may contribute substantially to anaemia among children was also consistent with other reports from developing settings.[31] Diarrhoeal diseases are reported to be associated with an increased production of cytokines, interleukin 6 and tumour necrosis factor alpha.[32] These cytokines are indicated to play a significant role in causing anaemia.[31] Repeated episodes of diarrhoea in children are also reported to lead to decreased nutrient absorption, due to injury of the small intestine mucosa.[33]

In our data, anaemia was also an independent risk factor for both IPIs and diarrhoea. In agreement with our findings, Levy et al., (2005) have reported that anaemia increases rates of infection in children. Furthermore, reports from several studies have indicated that anaemia can predispose people to infections by lowering host immunity.[35,36] In general, our findings strengthen the well-established notion that infection and malnutrition are intricately linked.[1,37,38]

No relevant trend of associations was observed between baseline demographics and the baseline and follow-up disease outcomes. Except for few instances, we did not find any of the measured demographic characteristics to be predictive of IPIs and malnutrition at follow-up. It is intriguing that history of anaemia did not predict risk of IPIs at baseline, yet it predicts risk at follow-up. To verify this finding, we double-checked the original laboratory results, the coding, and recordings in our data and found no errors. Parasitic infections cause long-term chronic infections by actively modulating their host's immune response.[39] Treatment of IPIs during the recruitment phase of the present study might have altered the established long-term mutual immune balance.[40] Any infection during the follow-up is a new infection that might induce immune reactions leading to chronic inflammation, blood loss, and red blood cell destruction.[19,20,41] This might have aggravated the severity of anaemia, among the already anaemic children and hence rendering them more prone to IPIs during follow-up.[34]

The observed significant preventive impact of hand-washing and nail clipping on intestinal parasite re-infection rates made us curious to explore whether the intervention effect noted in the whole cohort was homogenous across children with different backgrounds. Hand-washing with soap and nail clipping interventions consistently favoured reduction of intestinal parasite re-infection rates across each sub-group analysed. A significant difference in the effect of hand-washing was observed only for baseline parasitic infection status. Based on our data, it is possible to suggest that benefit from the interventions is likely to be more universal among the study groups. The observed increased benefit of hand-washing among children who were parasite-free at baseline should be interpreted with caution, although analyses were based on formal tests of interaction. Children were made parasite-free at recruitment and this might have affected our analysis at follow-up. The difference in effect for hand-washing and nail clipping between children who use pipeline and borehole water sources and those using wells and streams is quite large, but not significant because of the small number of children who use wells and streams.

Our data was based on a randomised controlled trial and our analysis was robust, where we used appropriate statistical methods for assessing heterogeneity of intervention effects among the levels of baseline with a statistical test for interaction. Further, the study design has enabled us to make an inference on the relationship between IPIs, anaemia and diarrhoea. However, the following limitation should be considered when interpreting the results of the present study: the study was powered to determine the overall effect of the interventions in the original randomised controlled trial. Our subgroup analyses might hence be underpowered to detect subgroup effects, unless the differences in treatment effects between subgroups would have been very large.

In conclusion, our findings emphasise that hand-washing with soap and nail clipping were efficacious in preventing intestinal parasite re-infection despite baseline differences and hence can be universally used as infection prevention interventions among school-aged children. Furthermore, intestinal parasitosis, anaemia and diarrhoea were independently associated. We urge for a holistic and integrated approach to break the vicious cycle of infection and malnutrition for the long-term health benefits of this population.

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Chapter 6

General discussion

Introduction

The studies presented in this thesis addressed three major issues. Initially, we investigated the prevalence and associated factors predicting intestinal parasitosis and under-nutrition among adult HIV/AIDS patients and school children. Data from the cross-sectional studies enabled us to determine the magnitude of the problem among these populations and provided the basis for research questions addressed in the subsequent studies. Second, we assessed the impact of simple hand hygiene interventions (hand-washing with soap at key times and weekly fingernail clipping) in the prevention of intestinal parasite re-infection, reduction of infection intensity and anaemia among rural school-aged children. Finally, we assessed associations between intestinal parasitic infections (IPIs), anaemia and diarrhoea among the children and further explored whether the impact of the hand-washing and nail clipping was similar across children of different demographic and disease backgrounds.

Magnitude and factors associated with intestinal parasitic infections among vulnerable populations

Our analysis revealed wide spread IPIs among antiretroviral-treated HIV/AIDS patients and school-aged children. This was consistent with other reports indicating high prevalence of IPIs among HIV infected populations[1,2] and school-aged children.[3] It is well recognised that a large proportion of people affected by HIV/AIDS live in deprived regions of the world[4] where the prevalence of IPIs is also remarkably high.[3]

In our cross-sectional studies, the prevalence of IPIs was lower among HIV/AIDS patients (56%) compared to school-aged children (72%). This finding was in agreement with the reports from a number of epidemiological studies which showed that children - school-aged children in particular, bear the greatest burden of infections with intestinal parasites compared to any other age group.[3,4] The disproportionate burden of infection among children has both behavioural and environmental bases, and makes children potential carriers of parasitic infections in a community.[5]

Worms, in particular soil-transmitted helminths (STHs), are reported to be the most prevalent parasitic infections and hence the major public health concern among marginalised populations of the developing world.[3,6,7] However, the prevalence of STHs was relatively low in our study populations. In both study groups (HIV/AIDS patients and school-aged children), infections with the protozoan gastrointestinal parasites, *E. histolytica/dispar,* were the most prevalent. The cestodes; *H. nana,* and the nematodes; *E. vermicularis,* were the next most common parasites identified among the school-aged children. Our data, although obtained from small populations, indicated a high prevalence of many other enteric parasites linked with poverty, which have not yet gained traction as neglected tropical diseases. Accordingly, our data advocates the need to include more parasites in the World Health Organization (WHO) Neglected Disease Initiative.[8] Initiatives concerned with controlling Neglected Tropical Diseases should consider including protozoan pathogens, such as *E. histolytica*, and other helminthes, like *E. vermicularis* and *H. nana* in impoverished settings where the parasitic infections are highly prevalent.

Our findings have also revealed that poor personal hygiene and sanitation practices contributed to the observed high prevalence of intestinal parasitosis and malnutrition in the study groups. Among the antiretroviral-treated HIV/AIDS patients, the habit of not washing hands with soap at critical times and living in households without latrines were significantly associated with increased risk of intestinal parasitosis. Although not very clear, associations between poor hygiene practice and parasitic infections were also observed among school children. However, poor personal hygiene and sanitation practices, particularly poor hand hygiene and the habit of open air defecation, were clearly associated with increased rates of anaemia and thinness in children.

Like other areas in the developing world, access to sanitation facilities in our setting was very low. Only 39% of the children in our study reported to use latrines at baseline (data not reported in the chapters). This was consistent with the 35% reported access rates to improved sanitation facilities in rural areas of the developing world.[9] Similarly, only 15% of the children in the present study washed their hands with soap at critical times at baseline. This was also in agreement with the mean prevalence estimates of hand-washing with soap (ranging from 13% to 17%) for low- and middle-income regions of the world, and with the estimated prevalence of 14% for Africa.[10]

Although hand-washing is a simple and cost-effective infection prevention measure[11,12], it is poorly practiced throughout the world.[12,13-16] Several complex and interdependent factors are reported to determine hand-washing compliance and hand hygiene behaviour in general.[12] Lack of water and soap, and inadequate provision of sanitation facilities are considered factors contributing to the observed poor hand-washing behaviour in children.[17] However, it has been reported that the mere presence of soap in the household does not improve hand-washing rates.[18] In poor settings, available soap in a household is often retained for other purposes, like washing clothes, dishes and other household utensils, and for bathing.[19]

Personal hygiene practice is also influenced by children's knowledge of and attitude toward personal hygiene and sanitation.[20] Children may perceive hand-washing as an additional assignment that takes time away from leisure activities, having meals and other activities of interest.[21] In Ethiopia, despite the increased political commitment to deliver hygiene and environmental health services[22], hand hygiene is given less attention. Existing efforts to promote good hygiene, including hand-washing, are not sufficient to bring mass behavioural changes. Much more attention is needed to alleviate the challenges of promoting correct hand-washing practices among at-risk populations such as young children and HIV/AIDS patients, with a need to ensure affordability and availability of hand-washing supplies and infrastructures.

Impact of regular hand-washing with soap and weekly nail clipping on child health

The results from the randomised controlled trial emphasise that hand-washing with soap at critical times and weekly finger nail clipping interventions are very efficacious in reducing intestinal parasite re-infection, the size of worm burden acquired after a successful treatment, and rates of anaemia among school-aged children. Given the notable impact of IPIs and malnutrition on school-aged children[3], the implication of these findings extends well beyond simple short-term health benefits among this age group. The heavy burden of IPIs among school-aged children[3,4] combined with nutritional deprivation have a negative impact on their long-term overall development.[23] Several deleterious effects, including retarded physical growth, anaemia and poor educational performance among children are significantly associated with IPIs.[24]

In regions of the world where the nutritional status of the community, and hence the child, is poor, even relatively light worm burden causes growth deficits.[25] Physical growth deficits during childhood have both short and long-term problems.[26] Among the short-term consequences are health problems such as high morbidity and mortality, child developmental shortfalls such as reduced mental and language development, and economic consequences due to increased health expenditures. Long-term consequences of growth defects in early life include reduced adult stature, reduced educational achievement, reduced work capacity and productivity in adulthood, and poor maternal reproductive outcomes at later years for females.[26,27]

Children with growth shortfalls enrol late in school and have higher dropout rates before completing primary school.[28,29] In deprived settings, this makes many children leave school without acquiring basic skills, which in turn, limit future productivity of the children and cause a continuous and significant loss of the limited educational resources that poor countries allocate for the provision of primary education. The health and economic implications of IPI prevention measures, including the contribution of hand-washing with soap and nail clipping, should also consider the burden IPIs impose on girls, who may become mothers in the future. Infants born to mothers with growth deficits during childhood are more likely to have low weight at birth and short stature as adults, contributing to the cycle of malnutrition in under-developed settings.[30]

The findings in the present study, which show a significant reduction in overall worm burden among children who washed their hands with soap and clipped their fingernails, demonstrate the efficacy of these interventions in protecting children from acquiring new worms following treatment. Establishment of each worm in the infected host is the result of a separate infection episodes as, except for few parasites such as *Strongyloides stercolaris* and *Capillaria* species, helminthes do not multiply within the same host.[30] A high worm load among infected individuals is therefore the result of repeated events of new infections.

Morbidity and related nutritional sequelae due to infections by helminthic parasites, especially the STHs, are related to the number of individual worms harbouring the gastro-intestinal tract of an infected person.[25] Prevention of repeated infestations as a result of our interventions has decreased the burden of worms among the children infected during the course of the trial. Such a reduction might have decreased morbidity due to parasitic infections and related nutritional deficiencies. Furthermore, the number of worms found in infected individuals determines the number of infectious stages shed to the environment (i.e. the infectiousness of the individual). The resulting reduction of worm burden due to the interventions, therefore, can be suggested to reduce environmental contamination and thus contribute to the reduction of infection transmission in the community.

Our randomised control trial (RCT) data demonstrate a significant reduction in the prevalence of anaemia among children who regularly washed their hands. The likelihood that anaemia will affect child development[31] makes it a serious public health concern for developing countries where anaemia is highly prevalent. Anaemia affects physical growth[32], cognitive development[33], and immune capacity[34,35] in children. Furthermore, studies have documented late school enrolment and poor educational achievement among anaemic children.[33]

Hand-washing at critical times lowered infection rates by parasitic worms. Based on these findings it is possible to suggest that the intervention lessens the chances of illness[36,37,38] and the development of chronic inflammation in infected children.[39] This can lead to reduced iron loss and increased nutritional intake, and hence might have reduced the prevalence of anaemia among children who have received the intervention.

In areas where infections with intestinal parasites and anaemia coexist, hand-washing with soap can make a difference by reducing nutritional tolls of infection, protect children from other developmental and intellectual defects, and reduce their risk of acquiring other severe diseases. School absenteeism due to illnesses was significantly lower (OR 0.13, 95% CI: 0.05 to 0.29) among children encouraged to wash their hands with soap (data not reported in the chapters).

Associations between intestinal parasitic infections, anaemia, and diarrhoea among school-aged children and the impact of hand-washing and nail clipping

We have demonstrated a clear relationship between IPIs, anaemia and diarrhoea among children. Although the notion of a vicious cycle of infection and malnutrition are well established[40-42], identification of specific associations are crucial in settings where infection and anaemia are common in order to design appropriate and holistic preventive programs, tuned to the prevailing conditions in the specific endemic setting. In the present study, we were not able to draw conclusions on the chronological sequence of the associations. From a public health viewpoint, we believe that our data have revealed important links between infection, malnutrition, and associated illnesses among the school-aged children, and in our view, no longitudinal study is needed to further establish their sequential relationships. The noteworthy point here is that proper hand-washing with soap breaks this link and prevents children from suffering the subsequent deleterious impacts of infection and anaemia.

The demonstrated protective impact of regular hand-washing and weekly nail clipping on intestinal parasitosis in our data prompted us to explore the universality of these findings among children with different demographic and disease characteristics. Overall, our analyses reveal that proper hand-washing with soap and weekly nail clipping is efficacious in children uniformly, despite their baseline risk differences.

Unlike our expectations, our analysis showed that regular hand-washing with soap at critical times benefited more children who were parasite-free at baseline compared to those who had a history of IPIs. This could be due to: (1) study participants were assessed and treated for IPIs at baseline and only parasite-free children were recruited for the trial; or (2) children with a history of infection at baseline may have several other risk factors in their respective environment that can predispose them to infection through other transmission routes (i.e. other than contaminated hands).

In general, hand-washing and nail clipping interventions seem to be efficacious for all individuals, provided that interventions are implemented properly with efficient follow-up. We believe that no other longitudinal study is needed to further verify the consistency of the benefits and we recommend hand-washing with soap to be a nationwide household intervention strategy.

Opportunities and barriers for wider implementation of hand hygiene interventions

Evidence from our data in the previous chapters reveals that IPIs and anaemia are a widespread public health problem in the study area. Furthermore, data from the RCT suggest potential health benefits of hand-washing and nail clipping interventions in reducing IPIs and anaemia among school aged-children. These findings can have considerable impact on the overall well-being of afflicted children in impoverished settings in the developing world.

Sustainable development of a society is founded on healthy and safe-guarded children. Similar to other countries with high fertility rates, a large proportion (43.5%) of Ethiopia's population comprises children under the age of 15 years.[43] Accordingly, due to concerns discussed above regarding the deleterious health and developmental effects stemming from IPIs and anaemia, tackling these problems is a high priority. Although long-term protection against parasitic infections is achieved through provision of safe water, adequate sanitary facilities and increased health access[44], they are unattainable in the short-term for poorer countries like Ethiopia. Our RCT shows that hand-washing with soap and weekly nail clipping are very efficacious public health interventions, which poor communities have at their disposal to combat infections and under-nutrition among children.

During our six month study, there were ample occasions to closely observe the opportunities and challenges for efficient implementation of hand-washing and nail clipping interventions at the household level. As in many developing communities, habitual and culturally embedded practices among children and households demanded intensive follow-up and monitoring. Implementation was particularly difficult among children, since they were reluctant to wash their hands at every important occasion and to follow correct hand-washing procedures. Focusing on mothers and older sisters within a household was essential in ensuring all members of the household continuously complied with the hand-washing protocol. We found that mothers and elder sisters play a vital role in the management of the household health. They not only help prompt the family, especially children, follow the hand-washing procedures, but they also make water available for hand-washing and sustain hand-washing facilities in the households. Our observations during the study period suggest that helping and enabling mothers to solve household health problems is the key to change long-established attitudes and behaviours around sanitation and hygiene within households.

Our experiences also suggest that the continuing public health problem of IPIs and anaemia in school-aged children is potentially solvable, and perhaps sooner rather than later. Hand-washing with soap and nail clipping can be implemented to wider populations. Proper hand-washing with soap and regular nail clipping strategies can be built into the primary health care systems and other existing health programmes. Education on proper hand-washing and nail clipping can also be incorporated into school curricula. Based on our findings, we urge for a holistic and integrated approach to break the vicious cycle of infection and under-nutrition for the long-term health benefits of the age group.

Provision of soap, nail clippers and other hygiene facilities by the government could create substantial opportunities in the struggle against infection and under-nutrition among children, considering that these basic and inexpensive measures have remarkable impacts on the health of children in the country. The challenge for effective implementation is the need for an intensive house-to-house follow-up, since provision of hygiene materials alone does not guarantee their proper use among poor communities.[19]

In Ethiopia, there are many opportunities that make house-to-house implementation feasible. The government has a policy of reaching inaccessible impoverished populations in the country through the Health Extension Program (HEP). Many thousands of Health Extension Workers (HEWs) are deployed to the most remote parts of the country. HEWs, recruited from the communities they serve, spend 75% of their time travelling from house-to-house and visiting families.[45,46] Hand-washing and nail clipping interventions and household follow-up can be incorporated into the HEP as hygiene and environmental sanitation is one of the major components of the health extension package at the household level.[45]

The activities of the Health Demographic Surveillance Site (HDSS) programme established in the study area and other parts of the country also involve intensive house-to-house visits to generate longitudinal data on vital statistics.[47] Implementation and follow-up of proper hand-washing and weekly nail clipping can be integrated with the HDSS activities. More importantly, a program recently implemented by the Ethiopian Federal Ministry of Health (FMOH), called the Health Development Army (HDA), organises and mobilises networks of one to five households within a community. Through this network, millions of volunteer community health promoters are mobilised nationally alongside the HEWs and they support families to adopt proper health behaviour.[48] Integration of house-to-house follow-up and hand-washing and nail clipping activities into the network could promote implementation and sustainability and may also enhance behavioural changes among the community. Ensuring the effectiveness and sustainability of a hand hygiene program requires firm political commitment and involvement of all relevant sectors to address the problem from multiple fronts.

Lessons learned

The RCT was carried out with a relatively small budget in a marginalised community of a low-income country. In our experience, conducting such studies is possible in resource-constrained environments. It was easy to find eligible participants, and achieve almost 100% participation. Human labour is relatively cheap, making house-to-house follow-up and other field activities possible. During the design phase of the study, we discussed whether to make the randomisation at district, school or household level, as we feared contamination. We believe selecting and conducting the randomisation at the household level was the correct decision. Throughout the study period, close observation found no signs of suspect contamination. Additionally, the majority of the infectious intestinal diseases arise within the home environment.[16] Implementation of the intervention (i.e. hand-washing with soap) at the household level by involving all family members potentially enhanced the effectiveness of the intervention.

Notably, the continuous household visits for promoting regular hand-washing and nail clipping appeared to change long-established hygiene and sanitation behaviours in the intervention households. During our stay in the study area, we observed considerable improvements in the cleanliness of the home environment and latrines, and waste disposal behaviour in the intervention households (Figure 6.1).

Figure 6.1 Differences in cleanliness of home environment among intervention (left) and control (right) households.

Conclusions

In sum, we assessed common health issues and related contributory factors among vulnerable populations in Ethiopia. Impacts of simple public health interventions on the prevention of IPIs and anaemia were assessed using a factorial RCT among rural school-aged children. For optimum protection of IPIs and improvement of the health and nutritional status of at-risk populations such as school-aged children in Ethiopia, we make the following major conclusive remarks and recommendations:

1. IPIs are of widespread prevalence among HIV/AIDS patients and school children in Ethiopia. Only non-opportunistic intestinal parasites were identified in both study groups, while peak prevalence of IPIs occurred among school children. The observed high prevalence of the protozoan parasites, *E. histolytica* and *G. lamblia*, among the participants may reveal the importance of faecal-oral transmission among the population groups.
2. Protein-energy malnutrition was highly prevalent among children. Prevalence of anaemia was of mild public health significance among this age group.
3. Relevant associations between poor personal hygiene and sanitation (in particular, unavailability of latrines and lack of hand-washing with soap) and intestinal parasitosis were observed among HIV/AIDS patients. Although patterns of association were not clear for all parasites identified, child personal hygiene and sanitation practice were also associated with IPIs. Among the school children, poor personal hygiene and sanitation was significantly associated with under-nutrition.
4. Proper hand-washing with soap at critical times and weekly nail clipping kept intestinal parasite re-infection rates and infection intensities low among the school-aged children. The interventions can hence prevent serious morbidity in children; an effect consistent with the rationale of deworming programs.[49] Furthermore, the interventions may reduce disease transmission and provide subsequent benefits for the community.
5. Proper hand-washing with soap at critical times reduced anaemia prevalence among the school-aged children.
6. Generally, hand-washing with soap at key times and weekly nail clipping prevented intestinal parasite re-infection among children, independent of differences in their baseline demographic characteristics. Benefits from the interventions are likely to be universal among the study groups. IPIs, anaemia and diarrhoea were linked and mutually reinforced one another in the study population; proper hand-washing with soap broke this vicious link.
7. There are major challenges in promoting correct hand hygiene practices in large communities among deprived populations, including changing long-established habitual and culturally embedded personal hygiene and sanitation practice, and ensuring affordability and availability of hand-washing supplies and infrastructures.

8. Ethiopia has many opportunities to implement and sustain regular hand-washing with soap and nail clipping on a larger-scale. Intervention programmes can be integrated into the existing community health programmes, such as the Health Extension Program, Demographic Surveillance Sites, and Health Development Army network, which reach inaccessible impoverished populations through house-to-house visits and outreach activities.
9. Randomised controlled studies are possible in resource-constrained environments even with limited budget allocations.

Future study plans

In order for communities to achieve optimal benefits from hand-washing with soap and nail clipping, cost effectiveness data may provide valuable information for their large-scale implementations. Further, in order to effectively implement the interventions, assessment should be conducted into indirect effects that may arise due to the interventions and changes in risk of transmission for a large population. We therefore plan to conduct future research works (1) on the cost effectiveness of the interventions; and (2) on extending the impact of the interventions to a) households other than intervention households in a community, and b) localities surrounding intervention areas.

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Summary

Summary

Chapter 1 presents the introductory background and premises upon which we laid and structured our research. It describes the rationale for undertaking our studies and generates the research questions based on the main pillars of this thesis: the magnitude and risk factors for intestinal parasitic infections (IPIs) and under-nutrition, protection against IPIs and under-nutrition through inexpensive and simple public health interventions, determining the association between IPIs, anaemia and diarrhoea and determining the universality of the effect of interventions among the study populations.

The chapter describes how IPIs and under-nutrition remain major health and socioeconomic challenges among underserved populations worldwide. Diseases caused by IPIs (both helminths and protozoa) and under-nutrition (both protein-energy malnutrition and micronutrient deficiencies) are of particular importance in terms of global burden, and their control has been recently recognised as a priority for achieving the United Nations Millennium Development Goals (MDGs).

IPIs and under-nutrition show similar geographic distribution, usually among poor populations which suffer from their combined effects. In such settings, infection and under-nutrition are highly interlinked. Children, particularly those of school-age, are heavily burdened by IPIs and malnutrition. They also serve as a source for the maintenance of IPI transmission. In endemic settings, children are continuously infected and re-infected with IPIs and this can eventually affect all aspects of their development. The number of IPIs affecting humans has increased following the emergence and expansion of the AIDS pandemic. A range of parasite species previously considered rare or zoonotic have become highly prevalent among humans and cause life-threatening illnesses in immunocompromised patients.

The high prevalence of infectious diseases and inadequate food intake has increased the prevalence of nutritional deficiencies among children in developing countries, resulting in health and development problems. Among the one billion people estimated to be affected by under-nutrition globally in 2010, 98% were from developing countries.

Micronutrient deficiencies also contribute significantly to the burden of under-nutrition. Anaemia is a widespread form of micronutrient deficiency affecting more than 2.6 billion people worldwide. Although causes of anaemia vary depending on the local conditions, iron deficiency is the most important contributing factor. IPIs (such as hookworms, ascaris and schistosomiasis), chronic infections (such as HIV, malaria and tuberculosis) and lack of other micronutrients can also lower haemoglobin levels and

increase the risk of developing anaemia. Children are among the most at-risk population for under-nutrition due to rapid growth stages and subsequent high nutritional requirements, combined with the increased risk of infection and poor dietary intake.

Despite the recent global interest in solving health problems of school-aged children, data on disease burden among these population groups are lacking in developing countries. The multi-factorial nature of health problems in this age group requires an integrated approach to solving the problem. The extent of health problems and contributing factors must be identified and addressed. Simple public health infection prevention measures also must be identified and integrated into the health intervention programs. An understanding of the infection and nutritional status of children and immunocompromised patients has far-reaching implications for health promotion and could provide valuable insight for decision makers in setting policy priorities and monitoring intervention programs.

Considering these issues, the studies in this thesis centred around three principle investigations. First, the magnitude of IPIs and associated risk factors were assessed among antiretroviral treated patients. Second, we investigated the prevalence and risk factors associated with IPIs, anaemia, and malnutrition in school-aged children. Third, based on the findings of these studies, we designed interventions that we believed could potentially alleviate risk factors for infection and under-nutrition, and assessed their impact in rural school-aged children. Based on the findings from this study, we further explored whether the impact of our interventions was similar among children despite differences in baseline demographic and disease characteristics, and assessed the pattern of associations between IPIs, diarrhoea, and anaemia among school-aged children.

The cross-sectional study conducted to assess the prevalence and associated risk factors for IPIs among antiretroviral-treated HIV/AIDS patients is presented in **Chapter 2**. A total of 384 patients receiving antiretroviral treatment (ART) were included in the study. Data on sociodemographic and personal hygiene and sanitation practice were collected from each participant using structured questionnaires. Parasitological surveys were completed on stool and urine specimens using direct microscopy and concentration techniques. Data on immunological profile and WHO disease staging was acquired from hospital records. The overall prevalence of intestinal parasitosis was 56% and no opportunistic gastro-intestinal parasites were detected. IPIs affected both genders equally except for Taenia species infections, whereby female participants had significantly less infections (OR: 0.34) than males. Intestinal parasitosis was associated with lower mean CD4+T-cell count and poor hygiene and sanitation practice. Lack of latrines and poor hand-washing with soap practice at critical times were significantly associated with higher *E. histolytica/dispar* (OR: 2.75 and OR: 2.67, respectively) and *G. lamblia* (OR: 2.08 and OR: 2.46, respectively) infections.

In our study, IPIs were widespread among the study group, with an overall prevalence higher than several similar studies from the country and other studies elsewhere. Non-opportunistic protozoan parasites, *E. histolytica/dispar* and *G. lamblia*, were the most frequent parasites encountered, followed by the zoonotic tapeworms, Taenia species. In this chapter it is highlighted that only extracellular gastrointestinal parasites were detected. Following the advent of highly active ART, the immune status of patients has improved, subsequently increasing protection against opportunistic infections and improving quality of life. IPIs may contribute to the morbidity of HIV/AIDS patients in endemic areas and their early identification could be important to improving the quality of life of patients under ART. Additionally, increased personal hygiene and sanitation can reduce faecal-oral transmission of IPIs among these populations.

Chapter 3 presents the results of a cross-sectional study on the risk factors associated with IPIs, anaemia and malnutrition among school children from urban and rural areas of northern Ethiopia. This study involved 600 school children from 12 primary schools. Demographic and socioeconomic data were collected using structured questionnaires uploaded onto a mobile phone using Datadyne Episurveyor software. Anthropometric data were collected by recording age, weight (to the nearest 0.1 kg), and height (to the nearest 0.1 cm) of the children. Parasitological analysis was carried out on fresh stool and urine specimens using direct saline wet mount, formalin ethyl acetate concentration and the Kato–Katz techniques. For helminthic infections, the total number of eggs detected on each slide was counted and the number of eggs per gram of faeces (epg) was calculated to determine egg burden. Urine specimens with positive screens for microhaematuria and those with gross haematuria were subjected to microscopic diagnosis for *S. haematobium* ova using urine sedimentation method. Haemoglobin concentrations of study participants were determined in finger prick blood using a HemoCue analyser.

Our analysis has shown a high prevalence of parasitic infections (72%) and energy-protein malnutrition, including stunting (35%) and thinness (34%) in our study populations. The protozoan parasite, *E. histolytica/dispar,* was the most frequently isolated parasite followed by the Cestodes - *H. nana*. No *S. haematobium* ova were identified. Egg concentrations for each identified helminthic infection were of light intensity. Anaemia prevalence in the study group was of a mild public health importance (11%). Although there were slight differences between urban and rural schools, problems of infection and under-nutrition were of the same magnitude. Overall, children with poor personal hygiene habits had higher prevalence of intestinal parasitosis. Similarly, the prevalence of anaemia and protein-energy malnutrition was higher among unhygienic children. Use of smart phone technology in the study improved data collection time and quality, and enabled us to develop simple data collection forms. Their potential use for field surveys in resource-limited settings is worth further investigation. Our study suggested the need for improved personal hygiene practices through increased access to hygiene facilities for hand washing and proper waste disposal. We identified a need for randomised trials to address the causal relationship between personal hygiene, parasitic infection, and malnutrition, which we subsequently conducted in Chapter 4.

Based on our findings from Chapter 3, we conducted a factorial randomised controlled trial among rural school-aged children to assess the impact of hand-washing with soap and nail clipping in the prevention of intestinal parasite re-infection, reduction of worm burden and anaemia prevalence. This study is described in **Chapter 4.** A study population of 367 school-aged children (aged 6-15) were randomised to one of four groups defined by a 2x2 factorial design: hand-washing with soap; nail clipping; hand-washing with soap and nail clipping; or standard habit and practice controls. Eligible children for the study were aged 6-15 years, screened negative for IPIs, were planning to continue residing in the same house for the study period, and had produced an informed consent signed by their parents or guardians.

Random allocation of study participants to the intervention and control arms was completed using computer generated random numbers. The principal purpose of the trial and assignment sequence was concealed from researchers recruiting the study participants. Laboratory personnel were blinded to group assignments and to the assessment outcomes. Following acquisition of a signed informed consent and before random allocation to intervention groups, a series of parasitological screening and treatment administration steps were conducted. Children who screened negative were randomly assigned to an intervention group and monitored for six months. At baseline, data on sociodemographics, personal hygiene and sanitation practices were collected using structured questionnaires. Finger prick blood was collected and haemoglobin concentrations were determined using a HemoCue analyser.

Interventions were implemented at the household level and randomly selected school-aged children within a household were classified as a study unit. Plain soaps were supplied to the hand-washing intervention households, who were encouraged to wash their hands with soap and water at critical times. New nail clippers were provided for the children allocated to the nail clipping intervention, and nail clipping was performed on a weekly basis by the fieldworkers. Children in the control arm were neither encouraged nor discouraged to wash and/or clip their nails. All households were visited with equal frequency on a weekly basis. Control households were frequently checked for the presence of soap for hand-washing and nail clippers during the weekly visits to check for contamination.

The trial concluded at the end of six months as scheduled. Stool specimens were collected and analysed by well-trained blinded laboratory personnel using direct saline wet mount, formalin ethyl-acetate concentration technique, and the Kato-Katz technique. Worm burden (infection intensity) for helminthic parasites was determined by calculating numbers of eggs per gram (epg) of stool. A child was classified as re-infected if an infection was detected by any methods used. After the trial, haemoglobin concentration was determined using the same procedure in Chapter 3. Haemoglobin readings were adjusted for altitude, and anaemia was defined for respective age and gender groups based on the WHO cut-off values.

The primary hypothesis of the study stated that hand-washing with soap and nail clipping would significantly reduce the prevalence of intestinal parasite re-infection rates and worm burden among the study population of school-aged children. Throughout the study period, only two children were lost to follow-up. Two others were excluded at the beginning of the study as they screened positive for intestinal parasitosis after successive treatments.

Regarding the study's primary outcome, only 14% (26/185) of children in the hand-washing with soap intervention were re-infected compared to 29% (53/182) of children in the control group (OR 0.32). Similarly, the prevalence of re-infection was lower among children in the nail clipping group, at 17% (33/189), compared to 26% (46/178) of children in the control arm (OR 0.51). Further, a significant reduction in worm burden was observed among children in the hand-washing group than the control group (mean epg =4.1 vs. 11.8, p=0.005), and nail clipping group than the control group (mean epg 3.4 vs. 12.6, p=0.001). At the end of the trial, 13% (24/185) of children in the hand-washing intervention were anaemic compared to 23% (41/182) in the control group (OR 0.39). There was also a reduction in the prevalence of anaemia among children of the nail clipping intervention; however the observed difference did not reach statistical significance.

The six-month hand-washing with soap and weekly nail clipping interventions demonstrated a significant reduction in intestinal parasite re-infection rates and the size of worm burden acquired after a successful treatment. A significant reduction in the prevalence of anaemia was also observed in children of the hand-washing with soap intervention. Given that school-aged children bear peak worm intensity in a community and that the size of the worm burden is the central determinant of transmission dynamics, proper hand-washing with soap and weekly nail trimming could have potential flow-on benefits to the community by reducing parasite transmission, in addition to immediate benefits in improving the health of children under consideration.

Our data showed that hand-washing and nail clipping were efficacious, however, our trial included rigorous follow-up and monitoring which involved high human resource investment. The long-established habitual and culturally embedded practices on personal hygiene and sanitation among children and households might require additional methods to influence behavioural changes and hence would make large scale implementations of the interventions more expensive. However, as labour is still very inexpensive in Ethiopia and other low income countries, national campaigns could be organised to implement hand-washing with soap at key times and weekly nail clipping through house-to-house education. Hand-washing and nail clipping interventions could also be integrated into existing community health programmes that reach inaccessible impoverished populations through house-to-house visits as their outreach activities. The next essential step should focus on implementing pragmatic studies that investigate the performance of the interventions under circumstances that more closely approach the real and usual conditions and developing additional more effective approaches to promote hand-washing with soap and nail clipping at larger scale. **Chapter 5** describes the pattern of associations between IPIs, anaemia and diarrhoea, and factors predicting IPIs and under-nutrition among school-aged children. We also explored whether the observed effects of interventions in the study population described in Chapter 4 could apply to children of different demographic and disease backgrounds.

The study design, intervention, follow-up, and laboratory investigation procedures are described in Chapter 4. The incidence of diarrhoea from all study households was documented by the field workers using a separate questionnaire during weekly household visits. The presence of diarrhoea was determined through corresponding with either the parents or the child. Two independent and trained data collectors took anthropometric measurements and the average recorded. Anthropometric measurements were converted into BMI-for-age Z scores using WHO AnthroPlus software. Children below -2Z scores were classified as thin.

Our findings demonstrated significant associations between IPIs, anaemia and diarrhoea among children. No trends in associations were observed between baseline demographic characteristics, IPIs, and malnutrition among the children. Only few potential effect modifiers were identified. Young child age was a significant predictor for anaemia (OR 1.82). Having young mothers predicted lower history of IPIs (OR 0.50). Living in small-sized families (OR 2.40), and history of anaemia (OR 2.08) and IPIs (OR 1.98) at baseline were significant predictors of higher risk of IPIs at follow-up. Hand-washing with soap at key times and weekly nail clipping were effective in reducing intestinal parasite re-infection rates in the study population. Our analysis suggests benefits from the interventions would likely be universal among the study groups.

Our findings in this chapter emphasise that hand-washing with soap and nail clipping can universally be used as infection prevention measures among school-aged children despite baseline demographic differences. Given that intestinal parasitosis, anaemia and diarrhoea have been independently associated, a holistic and integrated approach should be implemented to break the vicious cycle for the long-term health benefits of the population.

In the final chapter of this thesis, **Chapter 6**, we present the overall major findings, methodological considerations, and conclusions of the studies presented in this thesis. The major discussion points in this chapter are presented in three main areas (1) the prevalence and associated factors predicting intestinal parasitosis and under-nutrition among vulnerable populations, notably HIV/AIDS patients and school children; (2) the impact of simple hand hygiene interventions (hand-washing with soap at key times and weekly finger nail clipping) on the prevention of intestinal parasite re-infection, reduction of infection intensity and anaemia prevalence among rural school-aged children; and (3) identifying patterns of associations between IPIs, anaemia and diarrhoea among school-aged children, and an analysis of the impact of hand-washing and nail clipping on IPIs across different subgroups of children.

In this chapter we also discuss the widespread prevalence of IPIs and under-nutrition among vulnerable populations in the study area. We further illustrate that observed high prevalence rates were associated with poor hygiene and sanitation practices. The findings of our randomised controlled trial were also highlighted. Challenges and opportunities for large scale implementation of simple hygiene interventions were illustrated with due emphasis. The chapter closes with nine major conclusive remarks and recommendations, and a research plan that could be used for optimum prevention of IPIs and under-nutrition among school-aged children in low-income countries.

ማጠቃለያ (Summary in Amharic)

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ማብራሪያ፤ በዚህ የማጠቃለያ ፅሑፍ የተጠቀሱት አመተ ምህረቶች በሙሉ በአውሮፓውያን አቆጣጠር ናቸው፡፡ ይህ የጥናት ፅሑፍ (thesis) ስድስት ምዕራፎችን የያዘ ነው፡፡

ምዕራፍ አንድ፤ መግቢያ ሲሆን በዚህ ፅሑፍ ለቀረቡት የምርምርና የጥናት ፅሑፎች ሶስት ዋና ዋና መሰረታዊ መነሻ ሃሳቦችና ምሶሶዎች ማለትም፤ የአንጀት ጥገኛ ተህዋስያን (Intestinal parasitic infections) እና የተመጣጠነ ምግብ እጥረት (under-nutrition) ስርጭትና ተያያዥ መንስኤዎቻቸው፤ እነዚህን የጤና ችግሮች ለመቅረፍ የሚያስችሉ ቀላል የህብረተሰብ ጤና አጠባበቅ (public health) ትግበራ (interventions) ውጤት መገምገም፤ እንዲሁም በአንጀት ጥገኛ ተህዋስያን፣ በደም ማነስ (anaemia) እና በተቅማጥ በሽታዎች መካከል ያለውን ትስስርና እነዚህን የጤና ችግሮች ለመከላከል የተደረገው የህብረተሰብ ጤና አጠባበቅ ትግበራ (ጣልቃ ገብነት) በጥናቱ ተሳታፊዎች የሚኖረው ሁሉምአቀፍ (universality) ውጤት ወይም ጠቀሜታ በተመለከተ ለተደረጉት ጥናቶች መነሻ የሆኑ መረጃዎችና ግንዛቤ የሚያስጨብጡ ሃሳቦች የቀረቡበት ምዕራፍ ነው፡፡

ይሄው ምዕራፍ - የአንጀት ጥገኛ ተህዋስያንና የተመጣጠነ ምግብ እጥረት በአለም አቀፍ ደረጃ ተገቢና በቂ አገልግሎት በማያገኙ ድሃ የህብረተሰብ ክፍሎች ላይ ከፍተኛ የጤና፣ የማህበራዊና ኢኮኖሚያዊ ቀውስ ሆኖ እንደሚገኝ ይገልፃል፡፡ በአንጀት ጥገኛ ተህዋስያን - የአንጀት ትል (helminths) እና አሃዱ ህዋስ (protozoan) እንዲሁም በተመጣጠነ ምግብ እጥረት ማለትም ሁለቱንም - የገንቢና ሃይል ሰጪ ምግብ አናሳነትና (protein-energy malnutrition) የደቃቅ ንጥረ ነገሮች እጥረት (micronutrient deficiencies) አማካኝነት የሚከሰቱ በሽታዎች በዓለም ማህብረተሰብ ዘንድ ከሚያደርሱት ጉዳት አንፃር ሲታዩ ልዩ ትኩረት የሚያስፈልጋቸው ናቸው፡፡ ከዚህም የተነሳ ስለችግሮቹ በቅርቡ ግንዛቤ ተወስዶ የጤና ችግሮቹን መቆጣጠርም የአለም ሃገራት የሚሊኒየም ዕድገት ግብ (United Nations Millennium Development Goals – MDGS) ለማሳካት እንደ ቅድመ ሁኔታ ተወስዷል፡፡

የአንጀት ጥገኛ ተህዋስያንና የተመጣጠነ ምግብ እጥረት ስርጭት ተመሳሳይ የሆነ ጂኦግራፊያዊ ክፍፍልን ያሳያል፡፡ ከዚህም የተነሳ በአብዛኛው ጊዜ በነዚህ በሽታዎች ጥምር ጉዳት የሚደርሰው በድሃው የማህብረተሰብ ክፍል ላይ ስለሆነ በዚህ የማህበረሰብ ክፍል የአንጀት ጥገኛ ተህዋስያንና የተመጣጠነ ምግብ እጥረት እጅግ የተቆራኙ ሆነው ይገኛሉ፡፡ ህፃናት በተለይም ዕድሜያቸው ለትምህርት የደረሱ ህፃናት በነዚህ የጤና ችግሮች በእጅጉ የሚጠቁ ሲሆን የጤና ችግሮቹ በአካባቢው በቀጣይነት ለመኖራቸውም እንደ ምንጭ ሆነው የሚያገለግሉትም እነዚሁ ህፃናት የህብረተሰብ ክፍል ናቸው፡፡ ይህ ሁኔታ በሂደት በህፃናቱ እድገት ላይ ሁለንተናዊ የሆነ ጉዳት እንዲደርስባቸው ያደርጋል፡፡

የኤድስ ወረርሽን መከሰትን ተከትሎም የሰው ልጅን ለማጥቃት የሚችሉ ጥገኛ የአንጀት ተህዋስያን ቁጥር ጨምሯል፡፡ ካሁን ቀደም በተወስነ ስርጭት ይታዩ የነበሩ ወይም በእንስሳት ላይ ብቻ ተወስነው ይታዩ የነበሩ ብዙ ተህዋስያን አሁን የበሽታ መከላከል አቅማቸው በተዳከሙ ሰዎች ላይ በከፍተኛ ደረጃ እየተስፋፉና ለህይወት አስጊ ለሆኑ ህመሞች መንስኤ እየሆኑ ይገኛሉ፡፡

በታዳጊ አገሮች የነዚህ ተላላፊ በሽታዎች በከፍተኛ ደረጃ መኖርና በቂና የተመጣጠነ አመጋገብ አለመኖር በህፃናት ላይ ሁለንተናዊ የጤናና የእድገት ችግር ሊሆን ችሏል፡፡ በ 2010 በአለም አቀፍ ደረጃ በተመጣጠነ ምግብ እጥረት ተጠቅተዋል ተብለው ከተገመቱት አንድ ቢልዮን ሰዎች ውስጥ 98 በመቶ የሚሆኑት ከታዳጊና ከድሃ አገራት ሁነው ተገኝቷል፡፡

የደቃቅ ንጥረ ነገር (micronutrient) እጥረት ለአጠቃላዩ የተመጣጠነ ምግብ እጥረት (malnutrition) ችግር በከፍተኛ ደረጃ አስተዋፅኦ እያደረገ ይገኛል፡፡ የደም ማነስ በአለም አቀፍ ደረጃ በስፋት ያለ የደቃቅ ንጥረ ነገር የአመጋገብ እጥረት ችግር ሆኖ ከ2.6 ቢልዮን ሰዎች በላይ እያጠቃ የሚገኝ በሽታ ነው፡፡ ምንም እንኳን የዚህ በሽታ መንስኤዎች እንደየአካባቢው ሁኔታ የተለያዩ ቢሆኑም የብረት ንጥረ ነገር እጦት (iron deficiency) አንደኛውና ዋንኛው መንስኤ ነው፡፡ የአንጀት ጥገኛ ተህዋስያን (እንደነ ወስፋት፣ ጎንደራና ብልሃርዝያ)፤ ስር የሰደዱ በሽታዎች (chronic diseases) (እንደነ ኤች ኣይ ቪ፣ ወባና የሳምባ በሽታ) እንዲሁም የሌሎች ንጥረ ነገሮች እጥረት የደም ማነስ በሽታ አደጋን ሊጨምሩ ይችላሉ፡፡

ህፃናት ካላቸው ፈጣን የሰውነት ዕድገት ጋር ተያይዞ በሚኖራቸው ከፍተኛ የምግብ ፍላጎት፣ በተጨማሪም ከሌላው የሕብረተሰብ ክፍል በተለየ መልኩ ለተላላፊ በሽታዎች ተጋላጭነታቸው ጋር ተዳምሮ በተመጣጠነ ምግብ እጥረት ከፍተኛ ተጐጂ እንዲሆኑ ያደርጋቸዋል፡፡ ምንም እንኳን በቅርቡ ዕድሜያቸው ለትምህርት በደረሱ ህፃናት ላይ የሚታዩ የጤና ችግሮች ለመፍታት አለም አቀፋዊ ፍላጎት ቢኖርም፤ በታዳጊ አገሮች በሚገኙ ዕድሜያቸው ለትምህርት በደረሱ ህፃናት ላይ ያለው የበሽታ ጫና የሚያሳይ መረጃ በበቂ ሁኔታ አይገኝም፡፡ በዚህ እድሜ ክልል ውስጥ ላሉ ህፃናት የጤና ችግሮች በጣም በርካታ ከመሆናቸው የተነሳ መፍትሄዎቻቸውም የተቀናጀ ስራን ይጠይቃሉ፡፡ የጤና ችግሮቹ ስፋትና ለችግሮቹ አስተዋፅኦ በማድረግ ላይ ያሉትን ተጓዳኝ መንስኤዎች ተለይተው መታወቅና፤ ችግሮቹ በአግባቡ የሚወገዱበት ቀላል መንገድ በመለየት ከጤና እንክብካቤ ፕሮግራሞች ጋር ተቀናጅተው እንዲካተቱ መደረግ ይኖርበታል፡፡

በህፃናትና በሽታን የመከላከል አቅም ችግር ባለባቸው በሽተኞች ላይ የተላላፊ በሽታዎችና የተመጣጠነ ምግብ እጥረት ሁኔታና መንስኤዎችቻቸው ላይ ትክክለኛ ግንዛቤ መኖር፤ ለሚደረጉ የጤና ማስፋፋት እንቅስቃሴዎችና በውሳኔ ሰጪ አካላት ዘንድም አስፈላጊ ፖሊሲ በመቅረፅ ረገድ የተግባር ቅደም ተከል ለማውጣት እና እየተተገበሩ ላሉ የበሽታ መከላከል እንቅስቃሴዎች ውጤት ለመገምገም የሚኖረው ሚና እጅግ ከፍተኛ ነው፡፡

እነዚህን ሁኔታዎች ግምት ውስጥ በማስገባት፤ ይህ የጥናት ፅሑፍ (thesis) ማእከል ያደረገው በሶስት ዋና የምርምር ምሶሶዎች ላይ ነው፡፡

በመጀመሪያ: የአንጀት ጥገኛ ተህዋስያን ፀረ ኤች ኣይ ቪ መድሃኒት በመውሰድ ላይ ባሉ ታካሚዎች ላይ ያላቸው የስርጭት መጠንና ተዛማጅ መንስኤዎችቻቸው ላይ ዳሰሳ ተካሂዷል፡፡ **ሁለተኛ**፤ በተመሳሳይ መልኩ በትምህርት ላይ በሚገኙ ህፃናት የአንጀት ጥገኛ ተህዋስያን፣ የደም ማነስ (anaemia) እና የተመጣጠነ ምግብ እጥረት (under-nutrition) ስርጭትና ተዛማጅ መንስኤዎቻቸው ተፈትሸዋል፡፡

ሶስተኛ: የነዚህን ጥናቶች ውጤት መሰረት በማድረግ በጣም ቀላልና በማንኛውም ሰው መተግበር የሚችሉና እነዚህን የጤና ችግሮች ይቀርፋሉ ብለን ባመንባቸው የመከላከያ ዘዴዎች ብቃት በገጠር አካባቢ በሚኖሩና ዕድሜአቸው ለትምህርት በደረሱ ህፃናት ላይ ዳሰሳ ተደርጓል፡፡ በዚሁ የጥናት ውጤት ላይ ተንተርሰንም ይህ በኛ ጣልቃ ገብነት የተገኘው ውጤት በመላ በጥናቱ በተሳተፉ ህፃናት ላይ ተመሳሳይ ስለመሆኑና በህፃናቱ ውስጥ የታዩ የአንጀት ጥገኛ ተህዋስያን፣ የደም ማነስና የተቅማጥ በሽታዎች ያላቸው ትስስር ለመፈተሽ ተጨማሪ ጥናት ተካሂዷል፡፡

በፀረ ኤች ኣይ ቪ መድሃኒት ተጠቃሚ ከቫይረሱ ጋር በሚኖሩ ሰዎች ላይ የአንጀት ጥገኛ ተህዋስያን ስርጭትና መንስኤዎቹን በተመለከተ በአንድ በተወሰነ ጊዜ ገደብ ላይ አተኩሮ የተጠና የጥናት ዘዴ ውጤት **በምእራፍ ሁለት** ላይ ቀርቧል፡፡ በአጠቃላይ በነሲብ (random) የጥናት ተሳታፊዎችን በመምረጥ ዘዴ የተመረጡ 384 የፀረ ኤች ኣይቪ መድኃኒት ተጠቃሚ ህሙማን በጥናቱ እንዲካተቱ ተደርጓል፡፡ የጥናቱ ተሳታፊዎች ማህበረዊ ሁኔታ፣ የግል

ጤናና ንፅህና አጠባበቅ ልምድ በደንብ የተቀናበሩ መጠይቆችን በመጠቀም መረጃ የማግኘቱ ስራ ተካሂዷል፡፡ ጥገኛ የአንጀት ታህዋስያን ዳሰሳ ከያንዳንዱ ተሳታፊ የሰገራና የሽንት ናሙና በመውሰድ ተካሂዷል፡፡ የያንዳንዱ ተሳታፊ የCD4 መጠንና የአለም ጤና ድርጅት (WHO) የበሽታ እርከን (diseases staging) መረጃ ከሆስፒታሉ የበሽተኛ መዝገብ ላይ ተወስዷል፡፡

አጠቃላይ የአንጀት ጥገኛ ተህዋስያን ስርጭት 56 በመቶ ሲሆን፤ ከኮሶ ትል (Taenia species) በቀር ሁለቱም ፆታዎች በኩል ደረጃ በጥገኛ ተህዋስያኑ የተጠቁ ሆነው ተገኝቷል፡፡ ከፍተኛ የአንጀት ጥገኛ ተህዋስያን ስርጭትም ከዝቅተኛ አማካይ የCD4 ህዋስ መጠንና ከግል ንፅህና እጦት ጋር ቀጥተኛ ትስስር እንዳለው ጥናቱ አሳይቷል፡፡ በጥናቱ ከተካተቱ ሰዎች መካከል ከተገኙት የአንጀት ጥገኛ ተህዋስያን ውስጥ አሜባና ጃርድያ በተለየ መልኩ ከግል ንፅህና ጉድለት ጋር ቀጥተኛ ትስስር እንዳላቸው ታይቷል፡፡ ይህም ማለት፤ መፀዳጃ ቤት የማይጠቀሙ ከሚጠቀሙት፣ እንዲሁም እጃቸውን በውሃና በሳሙና የማይታጠቡ ከሚታጠቡት ጋር ሲነፃፀር መፀዳጃ ቤት የማይጠቀሙትና እጃቸውን በውሃና በሳሙና የማይታጠቡት የጥናቱ ተሳታፊዎች በሶስት እጥፍ ገደማ በአሜባ የመጠቃት ዕድል ነበራቸው፡፡ በተጨማሪም ከላይ የተጠቀሱ የግል ንፅህና ጉድለቶች የነበረባቸው ተሳታፊዎች የግል ንፅህናቸው ከሚጠብቁ የጥናቱ ተሳታፊዎች ሲነፃፀር ከሁለት እጥፍ በላይ ጃርድያ የተገኘባቸው ሆነው ተገኝተዋል፡፡

በጥናታችን በአጠቃላይ ሲታይ ከብዙዎቹ በአገሪቱም ሆነ በሌሎች ሃገራት ከተደረጉት ተመሳሳይ ጥናቶች የበለጠ ጥገኛ የአንጀት ተህዋስያን ስርጭት በጥናቱ በተካተቱት የፀረ ኤች አይ ቪ ቫይረስ መድሃኒት ተጠቃሚዎች ዘንድ ታይቷል፡፡ በዚሁ ጥናት ተጓዳኝ (opportunistic) ያልሆኑ የአንጀት ጥገኛ ተህዋስያን ብቻ የታዩ ሲሆን፤ በተለይም አሜባና ጃርድያ በአብላጫና በተደጋጋሚ በጥናቱ የተለዩ ተህዋስያን ሆነው እንዚህን ተከትሎ መሰረቱ የእንስሳት የሆነው የኮሶ ትል በብዛት ታይቷል፡፡

የፀረ ኤች አይ ቪ ቫይረስ መድሃኒት ግኝትን ተከተሎ ኤች አይ ቪ ቫይረስ በደማቸው ውስጥ ያለባቸው ሰዎች የበሽታ መከላከል አቅም እየተሻሻለ የመጣ ሲሆን፤ ተጓዳኝ (opportunistic) በሽታዎችን የመከላከል ብቃትም እየጨመረ ስለሄደ የነዚህ በሽተኞች ጤናማ ኑሮ ሁኔታም እየተሻሻለ መጥቷል፡፡ ጥገኛ የአንጀት ተህዋስያን በብዛት በሚገኝበት አካባቢ፣ እነዚሁ ጥገኛ ተህዋስያን የኤች አይ ቪ/ኤድስ በሽተኞች ጤናን ኩፍኛ የሚያናጉ ሲሆን የስርጭታቸውን ሁኔታ በጊዜ መታወቁ ደግሞ የፀረ ኤች አይቪ መድሃኒት ተጠቃሚዎችን ጤና ለማሻሻል የሚኖረው አስተዋፅኦ እጅግ ከፍተኛ ነው፡፡ በተጨማሪም ሰፋት ያለው የግል ንፅህና እንክብካቤ የተህዋስያኑ የመተላለፍያ ዕድል እንደሚቀንስ ጥናቱ ኣሳይቷል፡፡

<u>**ምዕራፍ ሶስት**</u>፤ ይህ ጥናት የአንጀት ጥገኛ ተህዋስያን፣ የደም ማነስና የተመጣጠነ የምግብ እጥረት ስርጭትና ተዛማጅ መንስኤዎቻቸውን ለመዳሰስ በትምህርት ላይ በሚገኙ የገጠር ህፃናት ላይ የተካሄደ ጥናት ነው፡፡ በዚህ ጥናት ከ12 ትምህርት ቤቶች የተውጣጡ 600 በነሲብ (random) የመምረጥ ዘዴ የተመረጡ ህፃናት ተካተዋል፡፡

የጥናቱ ተሳታፊዎች ማህበራዊና ኢኮኖሚያዊ መረጃዎች የተሰበሰበው በሞባይል ላይ በተጫኑ መጠይቆች አማካኝነት ነበር፡፡ በጥናቱ የተካተቱ ህፃናት የሰውነት እድገት መረጃ፤ የህፃናቱ እድሜ፣ ክብደት (ወደ 0.1 ኪ.ግ በማጠጋጋት) እና ቁመት (ወደ 0.1 ሳ.ሜ በማጠጋጋት) በመውሰድ ተሰልቷል፡፡ የአንጀት ጥገኛ ተህዋስያን ምርመራ የሰገራና የሽንት ናሙና በመውሰድ እና የተለያዩ የመመርመሪያ ዘዴዎች በመጠቀም ተካሂዷል፡፡ በምርመራው የተገኙት የአንጀት ትል (helminths) የበሽታ ጫና (infection intensity)፣ የተህዋስያን እንቁላል (eggs/ova) በመቁጠር በያንዳንዱ ለምርመራ በተወሰደ አንድ ግራም የሰገራ ናሙና (eggs per gram of stool - epg) ውስጥ የተገኙትን በማሰላት ተቀምጧል፡፡ የሽንት ፊኛ ቢልሃርዚያ (*Schistosoma haematobium*)

ስርጭት በሽንት ናሙናዎቹ በተደረገ ምርመራ ለመዳሰስ ተሞክሯል፡፡ በተጨማሪም ከጣት ደም በመውሰድ በጥናቱ የተካተቱ ህፃናት የደም ማነስ ሁኔታ ምርመራ ተደርጓል፡፡

በዚህ ጥናት፤ 72 በመቶ በጥናቱ የተካተቱ ህፃናት በጥገኛ የአንጀት ተህዋስያን፣ 35 በመቶ በአቀንጭራነት (stunting) እንዲሁም 34 በመቶ ህፃናት በመቀጨጭ (thinness) የተጠቁ ሆነው ተገኝተዋል፡፡ በአጠቃላይ በምርመራ ከተገኙት የአንጀት ጥገኛ ተህዋስያ ውስጥ አሜባ፤ ሃይሚኖሎፒስ ናናን (*Hymenolepis nana*) እና የአንጀት ቢልሃርዚያን (*Schistosoma mansoni*) አስከትሎ በህፃናቱ በብዛት የታየ የአንጀት ጥገኛ ተህዋስ ነበር፡፡ የሁሉም በጥናቱ ለተለዩት የአንጀት ጥገኛ ትሎች የበሽታ ጫና (infection intensity) በቀላል የበሽታ ጫና (light intensity) ደረጃ የሚመደብ ነበር፡፡ የደም ማነስ ስርጭትም በአለም ጤና ድርጅት (WHO) መስፈርት መሰረት በቀላል የማህበረሰብ ጤና ችግር የሚመደብ መሆኑ ጥናቱ አመልክቷል፡፡ በዚህ ጥናት፣ ምንም እንኳን አልፎ አልፎ ጥቂት ልዩነቶች የታዩ ቢሆንም ከገጠርና ከከተማ በጥናቱ በተካተቱት ህፃናት መካከል የበሽታና የስነ አመጋገብ እጥረት ስርጭት ላይ የጐላ ልዩነት እንደሌለ ጥናቱ ኣሳይቷል፡፡

በአጠቃላይ ሲታይ ዝቅተኛ የግል ንፅህና አጠባበቅ ልምድ የነበራቸው ህፃናት በከፍተኛ ደረጃ የአንጀት ጥገኛ ተህዋስያን የተጠቁ ሆነው ተገኝተዋል፡፡ በተመሳሳይ መልኩ ከፍተኛ የሆነ የደም ማነስ በሽታና የስነ አመጋገብ እጥረት (under-nutrition) ስርጭት የታየውም በነዚሁ የግል ንፅህና አጠባበቅ ችግር በነበረባቸው ህፃናት ላይ ነበር፡፡

በዚህ ጥናት የሞባይል ቴክኖሎጂን በመረጃ አሰባሰብ ስራችን ጥቅም ላይ በማዋላችን የመረጃ አሰባበሰብ ጊዜያችንና ጥራት አሻሽሎልናል፡፡ እንደዚሁም ለአጠቃቀም ምቹና ቀላል የሆኑ የመረጃ መሰብሰቢያ ቅፆችን ለመጠቀም አስችሎናል፡፡ የስማርት ሞባይል ቴክኖሎጂ በታዳጊ አገራት ውስጥ በሚደረጉ የመስክ ምርምሮች ላይ የሚኖረው ሚና የሚፈትሹ ተጨማሪ ጥናቶች አስፈላጊነትን ጥናቱ አመላክቷል፡፡

የተሻሻለ የግል ንፅህና አጠባበቅ ትግበራና ለንፅህና አጠባበቅ የሚጠቅሙ መገልገያዎች፣ ማለትም አዘውትሮ እጅን በሳሙና ለመታጠብና ለትክክለኛ የቆሻሻ (የሰገራ) አወጋገድ የሚረዱ መገልገያዎች አቅርቦት መኖር ወሳኝ መሆኑ ጥናቱ አመላክቷል፡፡ በተጨማሪም የቀላል የግል ንፅህና አተገባበር፣ የአንጀት ጥገኛ ተህዋስያንና የተመጣጠነ የምግብ እጥረትን በመቀነስ ረገድ ያለውን ሚና መፈተሽ አስፈላጊነት ጥናቱ ባመለከተው መሰረት የተደረገውን ጥናት በምዕራፍ አራት ቀርቧል፡፡

<u>ምዕራፍ አራት፤</u> በዚህ ምዕራፍ የቀረበው ፅሑፍ በምዕራፍ ሶስት በሰፈረው ጥናት ግኝት ላይ በመንተራስና የፋክቶሪያ ራንደማይዝድ የጥናት ዘዴን (factorial randomised trial)በመጠቀም እጅን በውሃና በሳሙና መታጠብንና በየሳምንቱ የእጅ ጥፍር መቁረጥን ዕድሜያቸው ለትምህርት በደረሱ ህፃናት ላይ በአንጀት ጥገኛ ተህዋስያን ዳግመ ጥቃት፣ በአንጀት ጥገኛ ተህዋስያን የበሽታ ጫና (infection intensity) እና በደም ማነስ ላይ ያላቸው የመከላከል ብቃት ላይ የተደረገ ዳሰሳ ውጤት ቀርቧል፡፡

በጥናቱ ከ216 ቤተሰቦች የተውጣጡ 367 ዕድሜያቸው ለትምህርት የደረሱ (ዕድሜያቸው ከ6-15 ዓመት የሆኑ) ህፃናትን ያካተተ ሲሆን የጥናቱ ተሳታፊዎችም ከኮምፒዩተር በተገኙ የነሲብ (random) ቁጥሮች መሰረት በአራት ቡድኖች በመደልደል ጥናቱ ተካሂዷል፡፡ በክፍፍሉ መሰረትም በቅደም ተከተል፤ እጃቸውን በውሃና በሳሙና የሚታጠቡ፣ ጥፍራቸው በየሳምንቱ የሚቆረጥላቸው፣ እጃቸውን በውሃና በሳሙና የሚታጠቡና ጥፍራቸው በየሳምንቱ የሚቆረጥላቸው፣ እና ምንም ዓይነት የእጅ ንፅህና ጣልቃገብነት (intervention) በማይደረግላቸው (control) ቡድኖች ውስጥ ህፃናቱን በመደልደል የእጅ ንፅህና የሚኖረው የበሽታ የመከላከል ብቃት ላይ የሙከራ ጥናት ተካሂዷል፡፡

በጥናቱ የተካተቱ ህፃናት ወይም በጥናቱ ለመሳተፍ ብቁ የነበሩ ህፃናት፤ ዕድሜያቸው ከ6-15 ዓመት የሆኑ፣ በጥናቱ መጀመሪያ ላይ በተደረገ የአንጀት ጥገኛ ተህዋስያን ምርመራና ህክምና ከአንጀት ጥገኛ ተህዋሰያን ነፃ የሆኑ፣ ጥናቱ በሚደረግበት ጊዜያት ሁሉ አሁን በሚኖሩበት ቤት ለመኖር የወሰኑ፣ እና ከወላጅ ወይም አሳዳጊ በጥናቱ ለመሳተፍ ፍቃድ ያገኙ ብቻ ነበሩ፡፡ የጥናቱ ዋና ዓላማና የጥናቱ ተሳታፊዎች የቡድን ድልድል በጥናቱ የሚካተቱን ህፃናት ለሚመርጡ ተመራማሪዎችና ለጥናቱ ተሳታፊዎች ግልፅ ሳይደረግ ቆይቷል፡፡ በተጨማሪም ይሄው የቡድን ድልድልና የጥናቱ ዓላማ የህፃናቱ ናሙና በመመርመር ለተሳተፉት የቤተሙከራ ባለሙያዎች ዘንድም ግልፅ ሳይደረግ ጥናቱ እንዲጠናቀቅ ተደርጓል፡፡

ጥናቱ ሲጀመር፣ በጥናቱ የተካተቱ ህፃናት ማህበራዊ ሁኔታ፣ የግል ንፅህና አጠባበቅ ልምድና የመሳሰሉትን መረጃዎች በመጠይቆች አማካኝነት ተሰብስቧል፡፡ ከያንዳንዱ ተሳታፊም የደም ናሙና በመውሰድም የደም ማነስ ምርመራ ተካሂዷል፡፡ እጅን የመታጠብ ሂደት በቤተሰብ ደረጃ የተከናወነ ሆኖ በተከታተይ ለስድስት ወራት ቤት ለቤት በመዘዋወር እያንዳንዱ የጥናቱ ተሳታፊ ህፃንና ቤተሰቡን በየሳምንቱ በመጐብኘት የጥናቱ ሂደት ተግባራዊነት ላይ ጥብቅ ክትትል ተደርጓል፡፡ የእጅ ጥፍር የመቁረጥ ሂደትም በየሳምንቱ በመስክ ሰራተኞቹ አማካኝነት ተከናውኗል፡፡

በውጤቱም፤ 14 በመቶ እጃቸው በሳሙና በሚታጠቡ ቡድን ውስጥ የነበሩ ህፃናት በአንጀት ጥገኛ ተህዋስያን የተጠቁ ሲሆን 29 በመቶ አጃቸው የማይታጠቡ ህፃናት (control) በነዚሁ ተህዋስያን የተጠቁ ሆነው ተገኝተዋል፡፡ በጥናቱ መሰረትም እጅን በተገቢው መንገድ በውሃና በሳሙና መታጠብ የአንጀት ጥገኛ ተህዋስያን ዳግመ-ጥቃት በግማሽ መቀነስ መቻሉን ለማየት ተችሏል፡፡ 17 በመቶ ጥፍራቸው የተቆረጠላቸው ህፃናት በአንጀት ጥገኛ ተህዋስያን ዳግመኛ ሲጠቁ 26 በመቶ ጥፍራቸው ያልተቆረላቸው ህፃናት በተህዋሳኑ ተጠቅተዋል፡፡ በተመሳሳይ መልኩም ሳምንታዊ የጥፍር መቁረጥ ሂደቱ የህፃናቱ በአንጀት ጥገኛ ተህዋስያን የመጠቃት ዕድል በአንድ ሶስተኛ መቀነሱን ጥናቱ አሳይቷል፡፡

በተጨማሪም እጃቸው በውሃና በሳሙና የታጠቡ እና ጥፍራቸው የተቆረጠላቸው ህፃናት፣ እጃቸው ካልታጠቡትና ጥፍራቸው ካልተቆረጠላቸው ህፃናት ጋር ሲነፃፀር እጅግ የቀነሰ የአንጀት ጥገኛ ተህዋስያን የበሽታ ጫና (infection intensity) እንደነበራቸው ጥናታችን አረጋግጧል፡፡ ይሄው ግኝት እጅን በውሃና በሳሙና መታጠብና ጥፍር መቁረጥ ከያንዳንዱ ህፃን ጤንነት አልፎ በማህበረሰብ ደረጃ የአንጀት ጥገኛ ተህዋስያን በሽታ በመቀነስ ረገድ ከፍተኛ ጠቀሜታ እንዳለው ያሳያል፡፡ ይህም የሆነበት ምክንያት ህፃናት በተለይም ዕድሜያቸው ለትምህርት የደረሱ ህፃናት፣ በከፍተኛ ደረጃ የተህዋስያኑ ተሸካሚና አስተላላፊ በመሆናቸው በጥናቱ የታየው የበሽታ ጫና ቅነሳ ህፃናቱ ያላቸውን የአካባቢ የመበከል አቅም ስለሚቀንሰው መላ ማህበረሰብበቡ በነዚህ ተህዋስያን የመጠቃት ዕድሉ አናሳ ይሆናል ማለት ነው፡፡

በተጨማሪም በተፈላጊው ወቅት እጃቸውን በውሃና በሳሙና ያልታጠቡት ህፃናት እጃቸውን ሲታጠቡ ከቆዩት ህፃናት ጋር ሲነፃፀር በእጥፍ በደም ማነስ በሽታ የተጠቁ ሆነው ተገኝተዋል፡፡ ባጠቃላይ እጅን በተፈላጊ ጊዜ በውሃና ሳሙና መታጠብና ጥፍርን በአግባቡ መቁረጥ የአንጀት ጥገኛ ተህዋስያን ዳግመ-ጥቃትን፣ የጥገኛ ተህዋስያን የበሽታ መጠንና የደም ማነስን በከፍተኛ ደረጃ የመከላከል ብቃት እንዳላቸው ጥናቱ አረጋግጧል፡፡

የቆየና ያልተስተካከለ የግል ንፅህና አጠባበቅ ልምድ በመኖሩ በዚህ ጥናት ቀጣይ የሆነ የቤት ለቤት ጉብኝት አስፈላጊ ሆኖ ተገኝቷል፡፡ ይሄም ሁኔታ ሰፊ የማህበረሰቡ አካላትን በማካተት ተመሳሳይ ጥናት ለማካሄድ እንደተግዳሮት ሆኖ ሊታይ የሚችል ጉዳይ ቢሆንም እንደ ኢትዮጵያ ላሉ በማደግ ላይ በሚገኙ አገራት በተነፃፃሪ ርካሽ የሆነ የሰው ጉልበት (cheap manpower) የሚገኝ በመሆኑ በዚህ ጥናት የተካተቱትን መሰል የበሽታ

የመከላከያ ዘዴዎች በቤት ለቤት ትግበራ ለማከናወን ይቻላል፡፡ በተጨማሪም በሃገሪቱ ውስጥ በቂ አገልግሎት በማያገኙት የህብረተሰብ ክፍሎች ዘንድ የጤና አገልግሎትን ለማዳረስ የታቀዱ በርካታ የጤና ፕሮግራሞች በመኖራቸው ብቃታቸው በዚህ ጥናት የተረጋገጠላቸውን ተመሳሳይ የበሽታ መከላከያ ዘዴዎች ከነዚሁ ፕሮግራሞች ጋር በማወሃድ በቀላሉ ለህብረተሰቡ ማዳረስ እንደሚቻል መገንዘብ ይቻላል፡፡ እጅን በውሃና በሳሙና የመታጠብንና ጥፍርን የመቁረጥ ተግባር በቀላሉና በተሳካ መለኩ ስፋት ላለው የህብረተሰብ ክፍል የሚዳረስበት መንገድ ማፈላለጉ ቀጣይና አስፈላጊ እርምጃ ይሆናል፡፡

ምዕራፍ አምስት፤ በአንጀት ጥገኛ ተህዋስያን፣ በደም ማነስ፣ በተቅማጥና በየመቀጨጭ (thinness) በሽታዎች መካከል የሚኖረውን ትስስር ዕድሜያቸው ለትምህርት በደረሱ ህፃናት ላይ የተደረገውን ጥናት የቀረበበት ፅሑፍ ነው፡፡ በተጨማሪም በዚህ ምዕራፍ፤ በምዕራፍ አራት ላይ የተገለፀውን የጥናት ውጤት፣ ማለትም እጅን በሳሙና መታጠብንና ጥፍርን የመቁረጥ ሂደት በሽታን በመከላከል ረገድ የነበራቸው ሚና በህፃናቱ መካከል ባሉት የግል ልዩነቶች ምክንያት ሳይሸራረፉ በሁሉም የጥናቱ ተሳታፊ ህፃናት ዘንድ ያላቸውን የብቃት ተመሳሳይነት ላይ የተደረገውን ዳሰሳ ውጤት የቀረበበት ምዕራፍ ነው፡፡ የዚሁ ጥናት ዘዴና ሂደት በምዕራፍ አራት የተገለፀ ሲሆን፤ ከዚህ በተጨማሪ በዚሁ ጥናት የተቅማጥ ስርጭት ሁኔታ (diarrhoeal incidence) ልዩ መጠይቅ በማዘጋጀት በየሳምንቱ በተደረጉት የቤት ለቤት ጉብኝት ወቅት ቤተሰቡንና ህፃናቱን በመጠየቅ ተሰንዷል፡፡ የህፃናቱ የመቀጨጭ (thinness) ሁኔታ ለመዳሰስ ሁለት የተላያዩ የመስክ ሰራተኞች የወሰዱት አማካይ የክብደትና የቁመት ልኮችን በመውሰድ የአለም ጤና ድርጅት ባዘጋጀው የኮምፒዩተር ፕሮግራም አማካኝነት እንዲወሰን ተደርጓል፡፡

የአንጀት ጥገኛ ተህዋስያን፣ የደም ማነስና የተቅማጥ በሽታዎች ቀጥተኛ ትስስር እንዳላቸው የጥናቱ ውጤት አመልክቷል፡፡ በዚህ ጥናት በህፃናቱ ማህበረሰባዊ ሁኔታ፣ በአንጀት ጥገኛ ተህዋስያን የመጠቃት ዕድልና በህፃናቱ የስነ ምግብ ሁኔታ መካከል ባጠቃላይ ቀጥተኛ ትስስር ያለመኖሩ ተመልክቷል፡፡ በተጨማሪም እጅን በአስፈላጊ ወቅት በውሃና በሳሙና መታጠብና የእጅ ጥፍርን በየሳምንቱ መቁረጥ እንደየህጻናቱ የማህበረሰባዊና የበሽታ ሁኔታ ሳይሸራረፉ የአንጀት ጥገኛ ተህዋስያንን ዳግመ ጥቃት ለመከላከል ያላቸው ብቃት በሁሉም በጥናቱ በተካተቱት ህፃናት ላይ ተመሳሳይ መሆኑ ጥናቱ አረጋግጧል፡፡

ባጠቃላይ በዚህ ምዕራፍ የቀረበው የጥናት ፅሁፍ የሚያረጋግጠው፤ እጅን በሳሙና መታጠብና ጥፍርን በአግባቡ መቁረጥ በሁሉም እድሜያቸው ለትምህርት ለደረሱ ህፃናት ላይ በሽታን ያለ ልዩነት የመከላከል ብቃት እንዳለውና የአንጀት ጥገኛ ተህዋስያን፣ የደም ማነስንና የተቅማጥ በሽታዎች ቀጥተኛ ትስስርን ተከትሎም እጅን በአስፈላጊ ወቅት በውሃና በሳሙና በመታጠብና በየሳምንቱ ጥፍርን በመቁረጥ ይህን ትስስር በብቃት መስበር እንደሚቻልና፣ ይህን የበሽታንና የተመጣጠነ ምግብ እጥረት ዑደት የሚያስቆሙና የህፃናትን ጤና በረዥም ጊዜ አንፃር ለመጠበቅ የሚረዱ ሁሉን አቀፍ የሆኑና የተቀናጁ የመከላከያ ዘዴዎች ተግባራዊ የማድረግ አስፈላጊነትን ነው፡፡

ምዕራፍ ስድስት፤ ይህ ምዕራፍ የጥናት መፅሓፉ የመጨረሻ ምዕራፍ ሲሆን በጥናቱ ውስጥ ለቀረቡት አራት የጥናት ፅሑፎች (ምዕራፍ 2-5) ዋና ዋና ግኝቶች ማጠቃለያ፣ የጥናት ዘዴዎቻቸው እና ያላቸው ትርጉም በሶስት ዋና ዋና ነጥቦች በማጠቃለል የተነተነ ምዕራፍ ነው፡፡

እነዚህ ሶስት ነጥቦችም፤ አንደኛ - በኤች ኣይ ቪ/ኤድስ ተጠቂ ሰዎችና በትምህርት ቤት ህፃናት ላይ ያላቸው ስርጭትና መንስኤዎቻቸው፤ ሁለተኛ - ቀላል የሆኑ የእጅ ንፅህና አጠባበቅ ዘዴዎች (በአስፈላጊ ወቅት እጅን በውሃና በሳሙና መታጠብን እና ሳምንታዊ የጥፍር ቆረጣን) ዕድሜያቸው ለትምህርት በደረሱና በገጠር በሚኖሩ ህፃናት ላይ የአንጀት ጥገኛ ተህዋስያንን፣ በነዚህ ተህዋስያን ምክንያት የሚከሰት የበሽታ መጠንና የደም ማነስን በመከላከለ ረገድ ያላቸውን ብቃት፤ ሶስተኛ - ዕድሜያቸው ለትምህርት በደረሱ ህፃናት ላይ በአንጀት ጥገኛ

ተህዋስያን፣ በደም ማነስና በተቅማጥ በሽታዎች መካከል ያለውን ትስስር እና ከላይ የተጠቀሱት ቀላል የእጅ ንፅህና መጠበቅያ ዘዴዎች በነዚሁ ህፃናት ላይ ያለ ልዩነት የሚኖራቸው ከበሽታ የመከላከል አቅም መዳሰስን የሚሉ ናቸው፡፡

በዚህ ምዕራፍ የአንጀት ጥገኛ ተህዋስያን እና የተመጣጠነ የምግብ እጥረት ችግር በጥናቱ በተካተቱት ተሳታፊዎች ዘንድ በስፋት ተሰራጭቶ መታየቱንና ይህ ከፍተኛ የበሽታ ስርጭትም እንደመንስኤነት ከግል የጤናና የንፅህና አጠባበቅ ጉለድት ጋር የተያያዘ መሆኑን በጥናቶቹ መረጋገጡን ያሳያል፡፡ በምዕራፉ የበነሲብና ቁጥጥር ባለበት (randomized controlled) የተደረገው ጥናታችን ግኝት በስፋት ቀርቧል፡፡ ቀላል የሆኑ የጤናና የንፅህና አጠባበቅ ተግባራትን በህብረተሰቡ ውስጥ በስፋት ለማካሄድ የታዩ ተግዳረቶችንና ያሉ ምቹ ሁኔታዎች በጥልቀት ቀርበዋል፡፡ በመጨረሻም ምዕራፉ ዘጠኝ የማጠቃለያ ሀሳቦችን በማስፈርና በማደግ ላይ ባሉ አገሮች በሚኖሩ ዕድሜያቸው ለትምህርት በደረሱ ህፃናትን ከአንጀት ጥገኛ ተህዋስያንና ከተመጣጠነ የምግብ እጥረት ጉዳት በበቂና በዘላቂ ሁኔታ ለመከላከል የሚያግዙ የወደፊት የጥናትና የምርምር ዕቅዶችን በማስቀመጥ ተጠናቋል፡፡

Resumen (Summary in Spanish)

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En **el Capítulo primero** se expone la introducción de este proyecto de investigación y se presentan los fundamentos sobre los que se han establecido la hipótesis y los objetivos del estudio. En este capítulo se describe la relevancia de de las infecciones parasitarias intestinales (IPI) en amplios grupos de población, especialmente niños, en Etiopía y gran parte de la población mundial. Los estudios realizados en esta tesis se han centrado en torno a tres cuestiones principales: 1) la caracterización de las infecciones parasitarias intestinales (IPI) y los factores de riesgo asociados en personas con la infección HIV/SIDA en tratamiento con antirretrovirales; 2) la caracterización de la prevalencia y factores de riesgo asociados con las IPI, la anemia y la desnutrición en los niños en edad escolar; 3) el diseño e implementación de un ensayo clínico para demostrar la eficacia de intervenciones de salud pública sencillas, como el lavado de manos con jabón y el recorte de uñas, en la reducción de las IPI y la desnutrición en niños en edad escolar.

El **capítulo segundo** se basa en el artículo publicado en la revista **International Journal of STD & AIDS** con el título "*Risk factors for intestinal parasitosis among antiretroviral treated HIV/AIDS patients in Ethiopia*".

En este primer trabajo hemos analizado la prevalencia y los factores de riesgo asociados a las infecciones parasitarias intestinales en personas infectadas con HIV/SIDA. Un total de 384 personas con HIV/SIDA en tratamiento antirretroviral (ART) fueron incluidos para este estudio. Mediante cuestionarios estructurados recogimos los datos sociodemográficos de este grupo de persobas. Los análisis parasitológicos en muestras de heces y orina se realizaron mediante técnicas de microscopía directa y de concentración, mientras que la información sobre el perfil inmunológico y la evolución de la enfermedad se obtuvo a partir de las historias clínicas hospitalarias. En este estudio, observamos que la prevalencia de las IPI era de un 56% (95% intérvalo de confianza 51% to 61%). Las IPI se asociaronde forma significativa a un menor recuento de células T CD4 + (p=0.002), pero no con el estadío de la enfermedad.

La escasez de letrinas y una mala higiene de manos se asociaron de forma significativa con una mayor prevalencia de infecciones por E. Histolytica /dispar (OR: 2,75 y OR: 2,67, respectivamente) y G. lamblia (OR: 2,08 y OR: 2,46, respectivamente). Los parásitos protozoarios no oportunistas como la E. histolytica / dispar y G. lamblia, fueron los parásitos más frecuentes encontrados, seguidos por las tenias zoonóticas.

En resúmen, la mala higiene personal y las deficientes condiciones sanitarias contribuyen de forma significativa a la alta prevalencia de IPI en pacientes con HIV/SIDA. Las infecciones parasitarias intestinales contribuyen a la morbilidad de los pacientes con VIH / SIDA y su cribaje rutinario en la clínica es acosnsejable para mejorar la calidad de vida de dichos pacientes.

El **capítulo tercero** se basa en el articulo publicado en la revista ***Pathogens and Global Health*** con el título *"Risk factors for intestinal parasitosis , anemia, and malnutrition among school children in Ethiopia".*
En este capítulo presentamos los resultados de un estudio sobre los factores de riesgo asociados a las infecciones parasitarias intestinales, la anemia y la desnutrición en los niños en edad escolar del norte de Etiopía.

Para este estudio, reclutamos de forma aleatoria a 600 niños distribuidos en 12 escuelas primarias en zonas rurales del norte de Etiopía. Los datos demográficos y socioeconómicos fueron obtenidos mediante cuestionarios estructurados utilizando aplicaciones específicas de smartphone. El análisis parasitológico en heces se realizó mediante los métodos de análisis directo en fresco, concentración y Kato-Katz. Las muestras de orina se utilizaron para el examen de huevos de Schistosoma haematobium. Asimismo, para las infecciones por helmintos, se analizó el número total de huevos y por gramo de heces. La concentración de hemoglobina se determinó mediante un analizador HemoCue.

Nuestros resultados indicaron que la prevalencia de parasitosis intestinal en este grupo de estudio era de un 72% (95% confidence interval (CI): 66-76%). El parásito protozoario, E.histolytica, fue el parásito más frecuentemente aislado, seguido de los cestodos - H. Nana. La prevalencia de anemia y retraso de crecimiento fué de un 11% % (95% CI: 8-13%), y un 35% (95% CI: 31-38%) respectivamente. Aunque hubo pequeñas diferencias entre las escuelas urbanas y rurales, los problemas de infección y la desnutrición fueron de la misma magnitud. Los niños con peores hábitos de higiene personal mostraron una mayor prevalencia de IPI, anemia y desnutrición.

En resumen, el uso de nuevas aplicaciones de teléfonos móviles para la recogida de datos en el estudio nos ha permitido un análisis de datos más eficiente y rápido. Nuestro estudio refuerza la necesidad de mejorar las prácticas de higiene personal en los niños promoviendo el lavado de manos generalizado y un mejor acceso a los servicios de saneamiento para reducir las infecciones parasitarias intestinales, la anemia y la desnutrición.

El capítulo cuarto, se basa en el articulo enviado a revisión a la revista **PLoS Medicine** con el título *Hand washing with soap and nail clipping: effect on intestinal parasitosis among school-aged children: a factorial randomised controlled trial".*

En este estudio evaluamos la eficacia de dos intervenciones de salud pública, el lavado de manos con jabón y el recorte de uñas, en la prevalencia e intensidad de las infecciones parasitarias intestinales en niños en edad escolar. Para ello planteamos un estudio factorial 2x2 con grupo control y aleatorizado. Se reclutaron un total de 369 niños, entre 6-15 años, seleccionados de forma aleatoria en 12 escuelas del norte de Etiopía. Todos los sujetos del estudio fueron monitorizados previamente para ver si presentaban IPI y asignados a alguno de los cuatro brazos del estudio: (a) lavado de manos con jabón; o (b) recorte de uñas; o (c) lavado de manos con jabón y recorte de uñas; (d) grupo control. A los seis meses de seguimiento, se realizó un examen de muestras fecales mediante técnicas de concentración directa y de Kato-Katz. La hemoglobina se analizó con un espectrómetro HemoCue.

Las objetivos primarios del estudio fueron la prevalencia e intensidad de la reinfección parasitaria intestinal. Nuestros resultados indicaron que un 14% (95% CI: 9% to 19%) de los niños en el grupo de lavado de manos con jabón se reinfectaron en comparación con el 29% del grupo control (95% CI: 22% to 36%) (OR 0.39). De forma similar, un 17% (95% CI: 12% to 22%) del grupo de recorte de uñas se reinfectaron en comparación con el 26% del grupo control (95% CI: 20% to32%) (OR 0.57). Asimismo, se observó una reducción significativa en la media de huevos por gramo en las heces, tanto en el grupo de lavado de manos con jabón (54 vs. 82 epg, p=0.001) como en el grupo de recorte de uñas (53 vs. 90, p=0.032). Un 13% (95% CI: 8% to 18%) de los niños en el grupo de lavado de manos presentaban anemia en comparación con el 23% (95% CI: 17% to 29%) en el grupo control (OR 0.49).

El lavado de manos y el recorte de uñas disminuyó de forma significativa la reinfección parasitaria intestinal y la intensidad de la carga parasitaria. Asimismo, el lavado de manos redujo la prevalencia de anemia. Estos datos sugieren la necesidad de promover de forma más eficaz y generalizada el lavado de manos y el recorte de uñas en niños en edad escolar.

El capítulo quinto se basa en el artículo en revisión con el título *"Associations between intestinal parasitic infections, anemia, and diarrhoea among school-aged children, and the impact of hand-washing and nail clipping"*

En este estudio se analizaron: 1) las asociaciones entre las IPI, la anemia y la diarrea en niños de edad escolar; 2) los factores que pueden predecir las IPI y la desnutrición; y 3) el impacto del lavado de manos y el recorte de uñas en niños con diferentes situaciones demográficas basales.

Los datos de este trabajo forman parte del ensayo aleatorizado descrito en el capítulo anterior, donde evaluábamos el impacto del lavado de manos y el recorte de uñas en la reinfección parasitaria intestinal. Las asociaciones entre las IPI, anemia y diarrea y otras características demográficas basales se analizaron mediante modelos estadísticos de regresión binaria y regresión logística múltiple. Nuestros resultados demostraron asociaciones significativas entre las IPI, la anemia y la presencia de diarrea. No se observaron tendencias en las asociaciones entre las características basales demográficas, las IPI y la desnutrición. La edad del niño fue un factor predictivo significativo de anemia (OR 1,82); Las familias de menor tamaño (OR 2,40), anemia (OR 2,08) y la presencia de IPI (OR 1,98) al inicio del estudio fueron predictores significativos de mayor riesgo de IPI tras la intervención.

En conjunto, estos resultados, sugieren que el lavado de manos con jabón y recorte de uñas son eficaces medidas de prevención de las infecciones parasitarias intestinales en los niños en edad escolar.

En **el capítulo sexto**, discutimos las principales aportaciones y lecciones del estudio, especialmente: (1) la prevalencia y los factores asociados a la infecciones parasitarias intestinales y la desnutrición en poblaciones vulnerables, en particular los pacientes con VIH / SIDA y los niños en edad escolar; (2) el impacto de simples intervenciones de higiene, como el lavado de manos con jabón y el recorte de uñas, en la prevención de la reinfección parasitaria intestinal, la disminución en la intensidad de la infección y de la prevalencia de la anemia entre los niños en edad escolar; y (3) la identificación de los patrones de asociación entre la IPI, la anemia y diarrea. Asimismo, al final de este capítulo, se resúmen las principales observaciones de nuestros trabajos y un grupo de recomendaciones para la mejor prevención de las infecciones parasitarias intestinales y la desnutrición en los niños en edad escolar en paises en desarollo.

Valorization

Valorization: Practical implications of findings

The findings of the studies in this thesis have significant implications on the health and development of school-aged children, as well as, the health of immunocompromised patients. Specific implications of the findings of each study, together with implementation and evaluation of simple hygiene and sanitation interventions in the prevention of IPIs and anaemia, are discussed in the respective Chapters. Here we summarise the overall implications of the major findings in this thesis.

Implication on the health of HIV/AIDS patients

The findings of these studies showed widespread prevalence of extracellular Intestinal Parasitic Infections (IPIs), and significant associations with poor personal hygiene and sanitation practices among antiretroviral treated (ART) patients. The negative contributions of IPIs to the rapid progression of diseases caused by HIV infection are long established. Identification of parasitic agents residing in the gastro-intestinal tract, and factors associated with high prevalence of IPIs among HIV/AIDS patients under ART, assist to optimise treatment and control measures that would improve the quality of life of these patients. The findings have highlighted the need for a better scheme of follow-up to improve the quality of care for HIV/AIDS patients under ART. Incorporation of regular stool tests and health education programmes that would help adopt preventive measures against IPIs for patients under care in ART programmes could be essential for the betterment of health services and hence, increase the likelihood of desired health outcomes in impoverished settings of the developing world.

Implication of prevalence and risk factors assessment in children

Children in developing settings are amongst the populations most affected by the deleterious health and developmental impacts of IPIs and under-nutrition. Both IPIs and under-nutrition share similar geographic distribution in the impoverished areas of the developing world and create a cyclic impact, ultimately contributing to defects in health, growth, and other forms of development among children. Prevention and control of IPIs (both helminthes and protozoa) and alleviation of under-nutrition (both micronutrient and protein-energy under-nutrition) are recognised as a priority for achieving the United Nations' Millennium Development Goals (MDGs). To be effective, interventions aimed at reducing the effects of infection and under-nutrition must be based on a proper assessment of the existing situation. Chapter 3 of this thesis investigated the prevalence and associated risk factors for intestinal parasitosis, anaemia and protein-energy under-nutrition among school children in northern Ethiopia. Findings in this chapter indicate a widespread occurrence of IPIs and under-nutrition among children. Poor personal hygiene and sanitation habits were generally associated with anaemia, thinness and IPIs. Children who had unclean hands were more often anaemic and thin. The findings emphasise the need for an increased access to clean water and latrines, and hygiene and sanitation communication activities to improve the health status of children living in endemic areas of the country.

The study used mobile phones to collect epidemiological data, which showed great potential as a more efficient tool for data collection in the remote field settings. Furthermore, the findings from the present study motivated the interest to design inexpensive and simple public health hygiene interventions and empirically assess their impacts in preventing IPIs and anaemia among school-aged children.

Implications of hand-washing and nail clipping on child health

IPIs and anaemia are of high prevalence among children in the developing regions of the world. School-aged children bear heavy worm burden from intestinal parasitic infections and are both the principal sufferers and source of transmission in a community. The results from the RCT indicated that hand-washing with soap at critical times and weekly fingernail clipping interventions were very efficacious in reducing intestinal parasite re-infection rates, the size of worm burden, and the prevalence of anaemia among the children. Given the notable health and developmental impacts of IPIs and anaemia on children, the implication of these findings extends well beyond simple short-term health benefits among these age groups. By reducing infection intensity, the interventions can prevent serious morbidity in infected children, aligning the effects of these simple public health interventions to be consistent with the rationale of deworming programs. Further, the efficacy in lowering infection intensity (worm burden) also suggests external benefit from reduced environmental contamination and hence reduced infection transmission in the community on a larger scale.

Long-term protection against IPIs and anaemia could be successfully achieved through public and economic development; however this is unattainable in a short period of time for poorer countries like Ethiopia. The current strategy of periodic treatment of at-risk individuals also has constraints due to systemic difficulties of drug provision, fear of increased potential of drug resistance and the short-lasting impact of treatment due to the frequent re-infection rate in endemic areas. Hand-washing with soap and nail clipping interventions, therefore have demonstrated to be an efficacious complementary measures that could help to lower the dependency on a 'drug only' approach and enhance sustainability of protective measures with available resources.

The important link between IPIs and anaemia reinforce the likelihood that proper hand-washing with soap can be used as comprehensive public health intervention to break this link and stop the deleterious health impacts of infection and anaemia in children.

Further, the substantial decline in intestinal parasite re-infection rates as a result of the interventions was seen among all children despite differences in their background.

The studies in this thesis were conducted in a resource-constrained country (Ethiopia) and have targeted vulnerable groups living in marginalised rural and urban areas. The findings presented in this thesis could be both useful and applicable to other children living in endemic areas throughout the world.

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With great respect and love!!!

Mahmud Abdulkader

Biography

Biography

Mahmud Abdulkader Mahmud was born in December 1st, 1971 in Mekelle, Ethiopia from his father Abdulkader Mahmud and his mother Zahra Mohammed Ali. He was grown up with his eight brothers and sisters. His father was a teacher who might have inculcated in him the interest of teaching that Mahmud has to date.

He attended his primary and high school education in Atse Yohannes Primary School and Atse Yohannes Comprehensive Secondary School in Mekelle, respectively. After completing his high school education, he joined Addis Ababa University, Ethiopia and he obtained his bachelor degree in Biology. In 1996/8, he lived in Addis Ababa, Ethiopia to pursue his post graduate study and obtained his MSc degree.

Mahmud began his professional career as a high school teacher. Since his recruitment as a governmental employee, he has served at different posts in different governmental institutions. Mahmud has served as instructor, department head and chief registrar in Mekelle University and other different private collages. He established and managed a regional health and research laboratory in the region, has served as a senior expert of HIV and STD in Regional Health Bureau and was employed in a pharmaceutical and medical supplies company at a position of a deputy general manager. During his second degree study, Mahmud was able to conduct research on HIV/AIDS in the then Ethio-Netherlands AIDS Research Project (ENARP). After he successfully completed his MSc, he has conducted some research works at Mekelle University by wining small scale research grants.

While serving in the Collage of Health Sciences, Mekelle University, Mahmud got a PhD scholarship in 2010 at the University of Alcala in Madrid, Spain sponsored by "Agencia Española de Cooperacion Internacional para el Desarrollo (AECID)', Spain. His PhD scholarship became a joint PhD program between the University of Alcala and Maastricht University, The Netherlands after about two years of his stay in Madrid. Mahmud's PhD research mainly focused on the prevention of intestinal parasitosis and under-nutrition in school-aged children in impoverished areas of developing countries using simple public health interventions.

Beyond his academic, research and work life, Mahmud enjoys reading books on life sciences, politics and psychology. He is delight in travelling, playing outdoor games, socializing, watching movies and listening to music. In his life time, he has been serving the people of his country and his passion and dream is to see healthy and safe-guarded children, where impacts imposed by infections and malnutrition among children come to an end.