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Assessment of Soil Transmitted Helminth Infection (STHI) in School Children, Risk Factors, Interactions and Environmental Control in El Salvador.

Sharad Suryakant Malavade
University of South Florida, sharad.malavade@gmail.com

Assessment of Soil Transmitted Helminth Infection (STHI) in School Children, Risk Factors,

Interactions and Environmental Control in El Salvador.

by

Sharad S. Malavade

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Department of Global Health
College of Public Health
University of South Florida

Co-Major Professor: Thomas Unnasch, Ph.D.
Co-Major Professor: Ricardo Izurieta, MD, DrPH.
Lakshminarayan Rajaram, Ph.D.
Linda Whiteford, Ph.D.
Boo Kwa, Ph.D.

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ABSTRACT

Background

Soil transmitted helminth infections (STHI) are important Neglected Tropical Diseases (NTD). The three main STHI are infections with *Ascaris lumbricoides, Trichuris trichiura* and hookworms. STHI have a significant effect on the growth and development of children. A national survey for STHI in El Salvador was carried out by Pan American Health Organization and Ministry of Health in 2012 in school children aged 8 years to 10 years. The survey collected data on age, gender, behavioral habits, and source of drinking water, type of toilet facility used and ecological zone of residence.

Aims

A) To determine the prevalence of STHI in El Salvador, assess the risk factors and risk factor interactions.

B) We also aimed to determine the efficacy of urea as a potential additive for inactivation of *Ascaris suum* in solar toilets.

Methods

A) Data from 1310 subjects was analysed for determination of prevalence of STHI in El Salvador. Risk factor assessment was done by chi-square test, unadjusted logistic regression and fully adjusted logistic regression. Risk factor interactions was tested on multiplicative and additive scale.

B) Urea was tested for efficacy in inactivation of *Ascaris suum* ova in 20 solar toilets. Under conditions of controlled pH and moisture, concentration of gas ammonia, peak temperature were

measured along with duration of treatment with urea to determine viability of *Ascaris suum* samples placed in the solar toilets.

Results

I) The prevalence of *Ascaris lumbricoides* in 8-10 year old school children is 2.75%, *Trichuris trichiura* is 4.1% and hookworm is 1.83%.

A) For *Ascaris lumbricoides* infection: Significant risk in individuals from volcanic chains and central depression compared to those from the mountains. Spring or well water when used as source of drinking water was associated with higher risk of infection when compared with piped water. Higher infection was also associated with open air defecation compared to use of septic tank or flush toilet. Use of sandals or no footwear was associated with a higher risk of infection when compared to use of closed footwear at all times.

B) For *Trichuris* infection: Coastal plains were associated with a higher risk of infection compared to the mountains while rural status was protective against infection. Spring or well water when used as source of drinking water was associated with higher risk of infection when compared with piped water. Use of sandals or no footwear was associated with a higher risk of infection when compared to use of closed footwear at all times.

C) For hookworm infection: Risk of infection was higher in individuals from urban regions. Spring or well water when used as source of drinking water was associated with higher risk of infection when compared with piped water. Use of sandals or no footwear was associated with a higher risk of infection when compared to use of closed footwear at all times. Poor handwashing was shown to be protective against infection with hookworm.

Significant risk factor interactions were identified for infection with each of the three soil transmitted helminths.

II) Urea as an additive at 1%w/w to feces tested in solar toilets showed an inactivation rate of nearly half the *Ascaris suum* ova samples. Fifty percent or higher inactivation rates were associated with ammonia gas concentrations of 109.5 ppm or higher and duration of treatment of 72 hours or higher.

Conclusions

Prevalence of STHI in 8-10 year old school children for 2012 in El Salvador is low. Significant risk factors for STHI in El Salvador are eco-epidemiologic zone, source of drinking water, type of sanitation, use of shoes behavior and urban status of place of residence. Use of urea for inactivation of soil transmitted ova in feces is a possible viable alternative.

CHAPTER 1:

INTRODUCTION

1.1 Purpose of the Study

Soil transmitted helminthes (STH) are one of the most common neglected tropical diseases (NTD).[1] STHs mainly cause nutritional imbalances the different infections affect humans at different times of their life. Commonly, they cause a decreased appetite leading to reduced food intake, lethargy, impaired digestion and absorption of nutrients from the diet and thereby poor growth and development of children. Some, like hookworms, are primarily responsible for blood loss leading to iron deficiency and iron deficiency anemia leading decreased working capacity of adults and impaired neurocognitive development of young children. [2]

Children in the school going age-group have the highest prevalence of STH infections worldwide.[3] The risk factors for the STH infection in children include behavioral factors and environmental factors. Behavioral factors like improper washing of hands after defecation and before meals are associated with a higher risk of STH infections. Also, environmental factors such as lack of access to safe drinking water and safe sanitation are also associated with increased risk of STH infection.[4-6]

Currently, mass drug administration is being used to treat STH infections in children. There are no vaccines currently approved for prevention of STH infections in humans.[7] However, drug treatment does not prevent recurrent infections in the presence of environmental and behavioral risk factors.[8]

Developing countries like El Salvador lack the necessary resources for provision of safe water and safe sanitation to their people. There is a need to develop safe sanitation technology that is socio-culturally acceptable, cheap and low resource intensive. Cruz et al. demonstrated the efficacy of soluble ammonia in human bio-solids for the inactivation of *Ascaris suum* eggs at the chemical and physical parameters present in solar toilets.[9] The use of urea as an ammonogen when mixed with human feces in the field would be an ideal low cost sustainable technology for provision of safe sanitation to developing countries.

The purpose of the current research is to determine the prevalence of STH infection in school children aged 8-10 years in the developing country of El Salvador and also determine the role of behavioral and environmental risk factors in the study population. Further, we test the field efficacy of urea as an additive to human feces for inactivation of *Ascaris suum* eggs in a developing country setting in El Salvador.

The dissertation has been divided into complementary subtopics: Chapter 1 describes the purpose of the study and the objectives; Chapter 2 includes the literature review; Chapter 3 is a manuscript titled 'Assessment of Prevalence, Risk Factors and Interactions for Soil Transmitted Helminth Infections in School Children in El Salvador'; Chapters 4 is a manuscript titled 'Piped water supplier of drinking water and Soil Transmitted Helminth infections in School Children in El Salvador'; Chapter 5 is a published paper titled 'A Novel Strategy for Environmental Control of Soil Transmitted Helminthes' and Chapter 6 is 'Conclusions and Recommendations'.

1.2 Hypothesis

The prevalence of STH infection in school children aged 8-10 years in El Salvador is low; the behavioral and environmental risk factors responsible for prevalence of STH infections in these children are not significant; and Urea (1%) inactivates *Ascaris suum* eggs in solar toilets.

1.3 Overall Objective

The overall objective is to determine the prevalence of STH infections in children aged 8-10 years in El Salvador and determine the role of behavioral and environmental risk factors in this prevalence; and evaluate the field efficacy of urea as an additive to human bio-solid for inactivation of representative STH ova (*Ascaris suum* ova) in solar toilets.

1.4 Specific Aims and Objectives

1.4.1 Aim 1: For each of the STH viz., *Ascaris lumbricoides*, *Trichuris trichiura* and Hookworm we have the following aims

- Aim1A: Determine prevalence in children aged 8-10 years in EL Salvador
- Aim1B: To determine association with personal, regional, water and sanitation, and behavioral risk factors
- Aim1C: To determine significant bio-behaviorally plausible interactions of risk factors
- Aim1D: Determine association between supplier of piped water and infection

1.4.2 Aim 2: Determine inactivation of *Ascaris suum* ova by urea (1%) added to feces in solar toilets in field environment in the community

1.5 References

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CHAPTER 2:

LITERATURE REVIEW

2.1 Water, Sanitation and Children's Health:

Along with air, water is the most essential element for human survival. All humans are dependent on water for their survival, particularly children. Children need water for consumption and washing, also using it for recreation. It is, therefore, of paramount importance that the water available to children be safe for consumption, washing and recreation. World Health Organization (WHO) and United Nations International Children's Emergency Fund's (UNICEF) Joint Management Programme (JMP) on Sanitation and Drinking Water defines 'Safe drinking water' as 'water with microbial, chemical and physical characteristics that meet WHO guidelines or national standards on drinking water quality'. The critical issue with respect to safe drinking water and children's health is the sustainable access to safe drinking water. WHO UNICEF JMP defines access to drinking water as when 'the source is less than 1 kilometer away from its place of use and that it is possible to reliably obtain at least 20 liters per member of a household per day'.[1] In 2010, the Millennium Development Goal (MDG) on drinking water was met. Despite this 768 million were still bereft of drinking water as of 2011.[2]

Lack of access to safe drinking water is responsible for diarrhoeal diseases in children which are the second most leading treatable cause of preventable death in children under 5 years of age killing about 760, 000 children in that age-group annually. Besides under-five mortality, diarrhoea is a significant cause of under-five malnutrition.[3] Availability and access to safe drinking water can significantly lower the proportion of diarrhoea cases. Improved water sources can reduce diarrhoeal cases by an estimated 21% and point of use disinfection of water can effect upto 45% reduction in diarrhea episodes.[4] Piped water source had a protective effect on children against intestinal parasitic infection as compared to stream water.[5] Dangour et al in their meta-analysis of the implementation of WASH (solar disinfection of water, provision of soap, and improvement of water quality) found a beneficial effect on the length of growth in children under five years of age for the relatively short intervention period of 9-12 months.[6] However, any lapse in the efficiency and vigil of water purification systems leads to diarrhea outbreaks even in countries with established safe water systems.[7]

The lack of adequate sanitation is responsible for the lack of availability of access to safe drinking water. The provision of facilities and services that ensure the safe disposal of human body wastes such as feces and urine is termed as sanitation. It also refers to the safe disposal and treatment and or processing of garbage and wastewater.[8]

There is significant burden of disease in communities that do not have adequate sanitation coverage. Improvement of the sanitation facilities and infrastructure is known to alleviate and reduce the burden of disease.[9] Clasen et al in their systematic review looked at interventions to improve excreta disposal to prevent diarrhoea and concluded that interventions to improve excreta disposal are protective against diarrhoeal disease.[10] Zeigelbauer et al in their 2012 systematic review found that the availability of sanitation facilities accorded significant protection against soil-transmitted helminthes with an odds ratio of 0.46-0.58. Use of the sanitation accorded varying odds ratios for protection against *Trichuris trichiura*, *Ascaris lumbricoides* and hookworm parasites.[11] The sanitation availability and use combined overall odds ratio for all three parasites was significant at 0.51 with a 95% CI of 0.44 – 0.61.

Studies have shown that children in the communities with inadequate sanitation do not achieve their full growth potential. Fink et al in their 2011 analysis to determine associations of water and sanitation across 70 countries found strong protective effects of high quality toilet facilities for neonatal, post-neonatal and child mortality risks.[12] They also found protective effects for episodes of diarrhoea and growth stunting. The access to high quality water had a protective effect on mortality in the 1 month to 1 year age group as they are the ones that are weaned. Lin et al in their 2013 study looked at the markers for the growth faltering environmental enteropathy in children in households in Bangladesh and the association with their domestic environment with respect to fecal contamination.[13] For their study they defined 'clean households' as those that had good water quality (median E.coli <10CFU/dl in water samples collected every three months for 2 years), improved sanitation (flush/septic/piped sewerage or a pit latrine with a slab and a water seal) and hygienic handwashing conditions i.e. a location only for washing hands with soap and water. They found that the children who lived in households that were clean had statistically significant lower levels of markers for environmental enteropathy that those who did not. Also, as they found that even if the clean environment household was surrounded by other households that did not qualify as clean households, the resident children still experienced a protective effect.[13]

Children that do not have access to safe sanitation, clean environment and safe water are also more likely to be hospitalized. This imposes an economic burden for the family and the public health infrastructure. The metabolic cost associated with illness translates into lesser than full achievement of the child's growth potential. Lander et al in their 2012 study of pre-school children in Brazil identified diarrhoea as the second commonest cause of hospitalization in their cohort of children, the first being respiratory illness.[14] They also found that children attending the private day care centers required repeated anti-helminthic therapy although less frequently compared to the public day care centers. This adds to the cost of healthcare although they did not quantify it. Taylor-Robinson et al in their 2012 Cochrane systematic review looked at the effect of deworming drug therapy in school children aged 16 years or less in community studies and did not find sufficient evidence to justify the contemporary practice of deworming programs as the evidence of consistent benefit on child nutrition, hemoglobin levels, school attendance and scholastic performance was not supported by reliable information.[15]

2.2 Behavior and Children's Health

Human behavior and practice has direct effects on human health both individually and communally. It has long been established that safe water and sanitation and hygiene habits are protective of human health.

The availability, access and proper use of safe sanitation is protective against the fecal oral transmitted infections. However, the caveat is that safe hygiene habits need to go along hand in hand with the safe water and sanitation facilities. Behavioral aspects that one needs to consider when looking at the fecal oral transmitted diseases in children are listed below.

2.2.1 Open Defecation

Open defecation has been linked to higher incidence of intestinal parasitoses in children when compared to children using either a private latrine at home or those who use a public latrine.[5] Barnard et al in 2013 did a cross sectional study evaluating latrine coverage and use among 20 villages in the state of Orissa three years after implementation of the Government of India's Total Sanitation Campaign.[16] They found that 62% of those households that had latrines had at least one member use it. However, only 47% of the individuals in those households used the latrine all the time of which about half were female. Thirty seven percent of the individuals living in households that had a latrine always practised open defecation. Additionally 5% of the individuals always defecated in the compound of the house and these were mainly the children while the remaining individuals only used the latrines sometimes or most of the times, practicing open defecation otherwise. Some of the reasons for non-use of the latrine included personal preference to open defecation, perceived inconvenience in using the latrine, incomplete construction of the latrine, lack of privacy, use of latrine for storage, broken latrine, choked up latrine outflow, distant latrines and difficulty in emptying the latrine pit. This study highlights the behavioral aspects that lead to failure of carefully thought of sanitary interventions.

2.2.2 Hand Washing After Defecation and Washing Prior to Eating Food, Availability and Affordability Of Water and Soap

Hand-washing with soap has been known to be effective in decreasing fecal-oral transmission of diseases.[17] Luby et al in their study published in 2011 found that washing hands prior to preparing food significantly reduced child diarrhea even when no soap was used.[18] They found that the children in households that had sub-optimal hand hygiene had lower rates of diarrhea compared to those living in households who had unacceptable standards of hand hygiene. Hand washing before eating has also been shown to protect against infection with Ascaris lumbricoides and Trichuris trichiura infection. The emphasis of hand-washing promotion programs stress on the five main occasions which are after defecation, after handling child feces or cleaning a child's anus who had defecated, before preparing food, before feeding a child, and before eating. Hand-washing is mostly practiced after defecation rather than food preparation or food consumption or feeding of the child and there is significant difference between the stated frequency and the observed frequency of hand-washing practice.[19, 20] Agustina et al found that the risk of diarrhea in children under 2 years of age was higher when the mother had poor hand-washing and food hygiene practices.[21] Nwaneri et al in their study in Nigeria found that hand-washing with soap and water after was associated with lower rate of infections with soil transmitted helminths in children.[22] Children engaged in hand-washing have been reported to have lower levels of absenteeism from school due to illness.[23, 24] Research shows the barriers to hand-washing behavior include, amongst other things, lack of awareness, cost and affordability of soap, prioritization of laundry, lack of control in access to soap, paucity of time in day to day work, cleanliness of the latrine and hand-washing facility.[23-28] However, policy research studies by the World Bank have failed to identify any demonstrable health benefits in terms of rates of diarrhea or soil-transmitted helminth infections in children under-five years of age between hand-washing intervention and control groups in India and Peru.[29, 30]

2.2.3 Eating Street Food, Eating Unwashed Food and Pica

Eating street food is the custom in nearly every nation of the world. Street food is attractive to patrons due to convenience, unique flavor profile and cheap cost.[31, 32] Food handling practices by the vendors are critical determinants of the infective potential of street food for transmission of gastrointestinal infections that can cause diarrhea, hepatitis or enteric parasitoses. Studies have shown that the incidence of diarrhea and gastro-intestinal diseases is higher in individuals that engage in consumption of street food versus control groups that do not. Also, consumption of certain types of street food carries a higher risk of disease transmission especially if it is composed of raw vegetables, fruits or meat. The holding method and time of the food post preparation is known to influence the microbial load in the food prior to sale. The street food vending sector is largely informal and unregulated in most of the countries.[33] The street food vendors are generally members of the lower socio-economic strata themselves and may lack adequate education, knowledge and means to ensure stringent standards of food hygiene.[32-35] They tend to use municipal water supply for preparing the food that may be contaminated with sewage; and also less likely to use soap detergents for washing of food preparation, mixing and serving containers to minimize cost and usage of water.[31, 32, 36]In the industrialized world hygiene practices and food handling practices of the chefs and servers are responsible for disease outbreaks.[37]

Eating of unwashed food is the other risk behavior that is associated with transmission of fecally transmitted pathogens. This is particularly true of green leafy vegetables like lettuce, spinach and celery; the root vegetables like carrots, radishes, beets and other vegetables like tomatoes, onions and cucumbers that are popularly consumed raw.[38, 39] Inadequate washing of the vegetables results in the consumption of the mud and dirt that can harbor fecal pathogens especially if human biowaste or animal biowaste is used as a source of manure/fertilizer.[39] Consumption of raw milk is also known to be a risk factor for serious diseases like Campylobacter infection and bovine tuberculosis. Consumption of raw meat can also, pose a risk as in street-side ceviche in Latin America. [40-42]

Pica is the consumption of substances that the consumer normally does not identify as food item. It occurs in some women during pregnancy.[43, 44] Children can develop pica during the early childhood especially if they have nutritional deficiencies, particularly iron.[45] Commonly pica in children tends to be consumption of mud, dirt or chalk. The consumption of dirt and mud is fraught with risk of increased risk of acquiring the soil transmitted helminthes.[22, 46-49] Also, fecal bacteria can be transmitted as young children tend to be less conscious of the voiding environments. This can be further enabled if the child routinely defecates in the house compound whilst also using the area as a playground.[16] Weidong et al found soil in and around houses and in the vegetable gardens to be contaminated with ova of Ascaris.[50]

2.2.4 Use of Footwear

Use of barrier protection. Self-protective behaviors like use of shoes that preclude direct skin contact with soil and any potential infective contaminants are known to be protective against infection with hookworms. Studies have shown that populations using closed footwear versus no or partially covering shoes have lesser incidence of hookworm infections.[51] A recent study showed no protective effect but it suffered from contamination, however, its observational data showed behavior change and a protective effect.[52] Similarly, protection of skin by use of protective creams for preventing cercarial penetration in regions endemic for schistosomiasis and swimmer's itch. The cream although effective is expensive and that can preclude its widespread use.[53] Another behavior is the use of insect repellant treated clothes and bed nets to protect against vector borne infections. This is well established for the case of tick-borne infections like Lyme disease, Ehrlichiosis and for malaria.[54, 55]

2.2.5 Recreational Exposure in Terrestrial and Aquatic Environments

Recreational exposure in terrestrial and aquatic environments. Apart from the domestic environment where the children and family are possibly aware of the risk of transmission of fecal-pathogens, other potential environments that are frequented by the children that put them at risk of fecal pathogens especially enteric parasites and soil transmitted helminthes are the play grounds, gardens and water bodies, whether natural or man-made. Jeffery and van der Putten in their 2011 European Commission Joint Research Center Institute for Environment and Sustainability report have divided the soil organisms causing human diseases in to two groups.[56] The first group is the euedaphic pathogenic organisms which are true soil organism with a pathogenic potential if they happen to infect humans and is mostly comprised of bacteria and fungi. The other group is the soil transmitted pathogens which are not true soil organisms. They are obligate pathogens that require a host animal to complete their life cycle, but can survive for extended periods of time in the soil. Table 1 gives the list from the book. A recent study found soil contamination rate of 68.1% with soil transmitted helminthes in samples taken from seven recreational parks in Abuja, Nigeria.[57] Blaszkowska et al in their 2013 study, found a seasonal variation in the positivity of soil samples for geo-helminth eggs from children's play areas in the city of Lodz, Poland.[58] Martinez-Bastidas et al in their 2014 pilot study detected pathogenic fecal micro-organisms on the hands and toys of children after playing on sidewalks and in public parks.[59] Sprenger et al in their 2014 found a significant association of positivity of soil and sand samples to geohelminth eggs and presence of cats and dogs and absence of fence around the sand box used by children to play.[60]

Aquatic recreation parks are also sites of transmission of fecal infective agents. There have reported outbreaks of diarrhea in children patronizing water parks.[61, 62] In a review published by Hlavsa et al looking at the outbreaks of human disease associated with recreation aquatic environments found that for both treated and untreated water environments, the leading causes were parasites.[63] Sixty-two of the 90 outbreaks with a known etiology associated with treated recreational water were caused by parasites, 18 were due to bacteria, eight due to chemicals and toxins and two were viral. For outbreaks associated with untreated water sources six out of 15 were due to parasites, four were due to bacteria, three were due to viruses, one was with multiple pathogens and one was by a toxin. Cryptosporidium accounted for more than 90% of the parasitic infections in outbreaks associated with treated water and remaining infections were by Giardia.

Exposure to warm water in a pond or lake exposes the children to the free living amebae and puts them at risk for infection with Naegleria fowleri.[64] Lakes and ponds are associated with infections with schistosomiasis, guinea worm disease.[63, 65, 66] Rivers are particularly important in the transmission risk of fecal transmitted pathogens as the run off from the ground that can be contaminated with fecal matter could end up in the river. There have been documented instances of sewage being drained into rivers.[67] The practice of open defecation next to the banks of the river also serves to compound the risk.[68]Marine waters are also source of infection.[63] A recent study identified swimming as the aquatic recreational activity most likely to result in ingestion of water.[69] Limited contact recreation like boating and kayaking on both the effluent impacted waters as well as waters designated for general use is associated with an increased risk of gastrointestinal illness.[70]

2.2.6 Handling Animals That Could be Source of Infective Agents

Handling animals that could be source of infective agents. Animals are a known source of infectious agents that cause human disease. About 60% of all cases of human infectious diseases are zoonoses. Animals can be biological reservoirs, hosts or carriers for infectious agents.[71] The transmission of infectious agents can be oral, respiratory, by inoculation or surface contamination as can happen by the animal licking the child. Domestic and peridomestic animals like dogs and cats are known to be carriers for various infective agents like toxoplasma, hookworms, bacteria and viruses. Pigs are known to be reservoirs for numerous infectious agents of human importance like Hepatitis E virus, influenza virus and *Ascaris suum*.[72-74] Genomic analyses now indicate the human round worm, *Ascaris lumbricoides* and the swine round worm *Ascaris suum* to be homologous enough to be thought of as strains of a single species.[75] The interaction of children with animals exposes them to the risk of acquiring these infections. Children come in contact with infective fecal material while petting the animals, playing with the tail and if playing in contaminated soils and sands. Infections that can be transmitted this way include soil transmitted helminthes, fecal coliforms, leptospirosis and hantavirus.[76, 77] The infectious agents originating from the domestic pet animals can be multi-drug resistant and transferred to the human handlers.[78] Interaction with an ill animal can transmit respiratory viral infections to children. Indeed, the swine flu virus can spread to humans through interaction with infected swine and vice versa.[74, 79] Bites and scratches can cause the innoculation of infective agents like the rabies virus, Pastuerella multocida, and Bartonella infections.[80-82] Handling the body fluids of animals with Brucellosis can transmit the infection to adults and children.[83] This is especially true in the farming communities where the children help out with the farming chores taking care of goats and sheep share the living space with animals during play and work.[84]

2.3 Life Cycle of Soil Transmitted Helminthes

Described below are the life-cycles of the most significant STH infections and how children can get infected with these parasites.

2.3.1 Ascaris Lifecycle (Figure 1.)

The adult worms reside in the intestinal tract. The mature fertile female worms release about 200,000 eggs per day that reach the external environment in the feces. Both fertilized and unfertilized eggs are uninfective at the time of release into the environment. Only the fertilized eggs develop embryo and become infective under optimal conditions of moisture, warmth, and shade in the soil. This can take anywhere from 18 days to several weeks. When the infective eggs are swallowed, the larvae hatch and invade the enteric mucosa and travel via the portal venous system and thence the systemic circulation to reach the pulmonary alveoli. Here they mature further residing for about a couple of weeks and then penetrate the alveolar walls to ascend the bronchial tree to the throat to be swallowed. Upon reaching back in the intestine the larvae mature into adults where they mate and the cycle continues. It takes about 2-3 months from the ingestion of eggs to the oviposition by the adult female worm. Adult worms can live for 1-2 years in the intestine. Figure 3 shows the lifecycle of *Ascaris* in graphic form.[85] In the case of *Ascaris*, the mode of infection is different from that of hookworms. In this case the infected eggs are ingested by the children through fecally contaminated food or water. Also, poor handwashing practices can lead to ingestion of infective ova after playing in areas contaminated with feces of infected host when toilet facilities are deficient and open air defecation is practiced.

2.3.2 *Trichuris trichiura* Lifecycle (Figure 2.)

The adult fertilized adult female releases unembryonated eggs that are passed out in the stool where they develop into a 2-celled stage which is an advanced cleavage stage after which they embryonate. It takes about 15-30 days for the eggs to be infective. When these eggs are consumed via contaminated food or water, the eggs hatch in the intestine to release larvae which mature in the colon. The worms mainly reside in the cecum and ascending colon with the anterior end of the worm buried into the intestinal mucosa.

After about couple of months, the adult females start oviposition and release 3,000 to 20,000 eggs per day. The worms live for about a year. Figure 4 shows the life cycle of *Trichuris trichiura* in graphic form.[86]

2.3.3 Hookworm Lifecycle (Figure 3.)

The hookworm eggs are passed in feces and in the soil under ambient conditions of temperature, moisture, shade and pH, the larvae hatch in a couple of days. These larvae are called the rhabditiform larvae, after growing in the feces and/or soil they develop into the infective filariform larvae in about 5-10 days and couple of molts. These filariform larvae are capable of surviving for about 3-4 weeks in favorable environment awaiting contact with the human host, when they penetrate the skin and are transported via the bloodstream to the lungs via the heart. There they penetrate the pulmonary alveoli into the airways and ascend the bronchial tree to be swallowed and end up in the small intestine where they reside and mature into adults. Adult worms attach to the intestinal wall resulting in blood loss of the host. Generally, the adults are eliminated in a couple of years although sometimes the infection can last for several years.

Sometimes the *Ancylostoma duodenale* larvae after dermal penetration can become dormant (in the intestine or muscle). Further, *Ancylostoma duodenale* infection can also occur via oral and transmammary routes. Figure 2 shows the lifecycle of hookworms in graphical form.[87] As we see, children can get infected when they walk or play in lawns, meadows or grounds where the soil is contaminated with feces from an infected human or animal host.

2.4 Complications Due to Enteric Helminthes

The STH cause various complications in the human population as discussed earlier. Important to recapitulate are the growth retardation of infants and children, impaired neurocognitive development, anemia and iron deficiency, decreased work capacity, macro- and micro-nutrient deficiencies, intestinal obstruction, hepatobiliary involvement in ascariasis and rectal prolapse in trichuriasis.

2.5 Geohelminth Infections and Socioeconomic Status (SES)

Socioeconomic status is defined as a measure that conceptualizes the social standing of an individual, family or group calculated based on income, education and occupation.[88] Generally SES is considered to be three tiered- low, middle and high. Education is considered a stable indicator of SES. For health related studies, the educational attainment of the mother is considered a good proxy for the evaluation of children's health as higher maternal educational levels are associated with better health outcomes[89-91] Better income is linked with better access to good quality food, access to health care, less mental stresses about meeting monetary emergencies. On the other hand, having higher education is linked with making more money as income and socioeconomic benefits.[92, 93] Individuals with lower levels of education earn lesser than those with higher levels of education.[94-96] Occupation has been shown to influence health status and outcomes. Low prevalence of intestinal helminthes was found by a 1992 study done by Marnell et al in Juba, Sudan amongst refugees looking at previous occupation.[97] They found that medical workers followed by the white collar workers had the lowest prevalence of STH infections, while blue collar workers and agriculturists had much higher prevalence. Other than these three, there are other factors that influence SES such as race, ethnicity, gender, sexual orientation which are important based on the community in context. Kightlinger et al found aggregations of *Ascaris lumbricoides* infections were associated with gender, type of housing ethnicity and agricultural activities.[98]

SES plays a very vital role in health of an individual and population. An individual from a low SES is less likely to have access to health promoting infrastructure such as safe sanitation and safe water, preventive public health services, access to facilities for physical activity such as playgrounds and gymnasia, food and healthcare facilities and services.[99]

The health of a population is an indicator of the past investments of the country in health.[93] The low resources of the developing country and the low income of the individuals make access to health beneficial infrastructure a challenge.[100] Low national resources lead to concentration of health promoting infrastructure to urban areas. For e.g. San Salvador being an urban region has better safe sanitation coverage that the rural parts of the El Salvador.[1, 101] However, being an urban region does not equate to high SES status as most of the people in the urban region are migrants from the hinterland who seek employment and a little income leading to crowding and increased demand on the existing infrastructure. In cities like Mumbai, there are a significant number of homeless individuals, individuals living in shanty towns with no access to safe sanitation.[102]

Similarly, the access to safe water is limited in a developing country either due to no infrastructure on the ground to supply each and every household with safe water, or due to ageing infrastructure experiencing wear and tear along with sabotage by those with no access to water supply who break into pipelines to access water thereby compromising the quality of water supplied downstream.[103, 104] Studies have shown that individuals with higher SES are more likely to have access to safe water and safe sanitation, but also, have the knowledge of disease transmission due to better educational levels and income that enables them to afford use of soap for hand washing every time before eating or making food and after using the toilet or handling pet animals like dogs and cats, water purifying devices and fuel to heat water.[98] Thus their risk of infection with geohelminthes is lower compared to those individuals in the lower SES strata. However, if they do not practice good hygiene behavior and consume contaminated raw fresh produce, their risk for infection with geohelminthes is increased. Izurieta et al in their 2001 survey looking at the sanitation behavior and environmental conditions in desiccating latrines in El Salvador found that 35% of the rural households fell into the low SES category compared to 40% of the urban households.[105] However, Pullan and Brooker in their 2012 study found no socioeconomic correlates to soil transmitted helminthes transmission when looking at national level, but also identified that countries with little or no STH infection had GDP of US$20,000 or higher.[106]

2.6 Ecology, Climate Change and STH

Ecology plays a significant role in the maintenance and transmission of soil transmitted helminthes. The survival of the STHs in the environment is dependent on the ambient temperature, pH of the soil, moisture of levels of the soil. Given this scenario, they thrive in warm, moist climates with adequate rainfall to maintain the optimal soil moisture level preventing desiccation. Nearly all the countries in the tropical belt in Asia, Africa are endemic to STH infections. Given this scenario, the STH parasites thrive in the tropical regions of the world and some of the temperate regions that abut the tropical regions.

Xu et al in their national survey found that all the three STH of importance i.e. *Ascaris,* Hookworms and *Trichuris* had had highest prevalence in tropical, subtropical, South temperate, meso-temperate and north temperate regions in that order of decreasing prevalence. [107] The difference between the prevalence was also very significant. Prevalence of *Ascaris* was 59.7% in the tropical regions while it was 5.4 % in the northern temperate regions i.e. a 10-fold difference. With *Trichuris trichiura*, the difference was even more striking. The prevalence was 50.3% in the tropics compared to 0.5% in the northern temperate regions reflecting a 100-fold difference. The most dramatic difference in prevalence was seen for hookworms. The tropics had a prevalence of 53.1% while northern temperate had zero percent prevalence. They reported a positive correlation between moisture and rainfall and prevalence of STH infections while for temperature, altitude and latitude they found a negative correlation.[107]

The egg forms of the some STHs can survive desiccation such as those of *Ascaris*, however, the eggs of hookworms are fragile and it is the larvae that are the infective forms. The larvae bury 1-2 mm below the surface of the soil to avoid the direct heat of the sun and destruction by heat and drying. While the tropical climates are most favorable for the parasites, the parasites can survive in the temperate climates as well. Studies have shown that in the temperate climate, the parasite eggs can survive over the winter to continue transmission in spring and summer thereby maintaining transmission.

The prevalence of STH infections is found to be lower in the mountains than in the plains at sea level due to the altitudinal effect on ambient temperature. Also, the rains in the mountains tend to wash away the eggs with the soil to the lower regions. Mabasso on their study on the effect of climate and soil type on prevalence of hookworm in South Africa reported that place that were ≤ 150 m from sea level i.e. in the sandy coastal plains were associated with a higher prevalence of hookworm infection that those above i.e. the mountainous regions.[108] The type of soil was also found to be determinant on the prevalence of hookworm infection. They found that having sandy soil was associated with higher prevalence than clayey soil.

The soil particle size until it is less than a third the length of larva helps with faster larval locomotion due to better traction and higher rigidity of the soil particles helps with the resistance necessary for locomotion due to undulating movements. The moisture level of the soil have an effect on the locomotion and breathing of the larvae. The higher the moisture level, lesser are the open pores in the soil affecting the level of oxygenation as well as the lack of open space affects locomotion and transmissibility. Factors that influence the mobility of hookworms determine their abundance as hatching, movement through soil and invasion of the host are important parts of its lifecycle.[109] These places had higher rainfall and soil moisture level. Beaver reported on the survival of *Ascaris* ova in different experimental soil conditions.[110] The eggs survived and matured faster becoming embryonated when in clay soil compared to sandy soil under shade. In sandy soils exposed to direct sunlight, the ova survived poorly. After three months they could find much more numerous eggs in the shaded clay plot compared to exposed sandy plots. Also, numerous embryonated eggs could be recovered from the shaded clay plot despite periods of intervening drought and heavy rainfall. Rain splashing caused the eggs to be transported and deposited on vertical surfaces including vegetation. Also, due to the mechanical sorting effect due to surface agitation, in sandy soils, the eggs concentrated in the most superficial layer of sandy soil, whilst being buried deeper in clay soil. This protects the eggs from desiccation and solar exposure thereby enhancing their chances of transmission to a susceptible host. During the dry season he found viable *Ascaris* near refuse deposited peri-domestic habit due to retention of moisture and dampness. He concludes saying that clays soil favors the development and maturation of *Ascaris* while sandy soil does the same for hookworm larvae.[110]

Climatic events like high temperatures and rainfall affect the soil habitat of the infective forms of STH and thus have an impact on their prevalence. Also, the socio-economic effects of climatic events can adversely impact agricultural practices and outputs causing lower access to food crops. This can indirectly influence STH prevalence rates due to access to nutrition and healthcare indirectly.[111] Climate changes can affect the complex interaction between parasite, host and environment thereby altering the parasite ecology. This can result from effects on susceptible hosts due to effects on their health or by their migration, infection pressure dependent on the abundance of parasite, their target hosts and parasite fecundity, prevalence and intensity of parasites due to direct and indirect effects. Weaver et al in their 2010 paper summarized the effect of different climatic parameters that have an effect on the development of geohelminths. For hookworms, increased temperature would reduce the time to infective for hookworms from time of discharge in feces due to faster development of the L1 and L2 larval stages, ambient relative humidity enhances survival of the larvae in soil while loss of humidity adversely affects larval soil survival. For *Ascaris* and *Trichuris*, increased temperature accelerates the time to embryonation shortening the time to infectivity however, beyond critical levels it is associated with death of the ova. Increased rainfall is beneficial for the infective forms of all STHs by safeguarding them against desiccation. However, in excess it leads to a decrease in larval hatching and development probably due to near zero oxygen tension in water in the soil interstitial spaces than the air water interface in in the interstices that the larvae inhabit. Decreased rainfall on the other hand reduces embryonation and larval release. Increased relative humidity which is dependent partly on the precipitation of rain enhances survival of the eggs.[109, 111]

The temperature sensitivities of the different parasites reflect the evolutionary survival strategies of STHs. *Ancylostoma duodenale* ova have an optimal temperature for hatching that is lesser than *Necator americanus* thereby enabling it to have a lower survival threshold temperature. Contrastingly, in Cameroon, in Central Africa, the upper threshold of survival for hookworm is 40-47^{0}C which is lethal for *Ascaris* and *Trichuris*. Additionally, *Ancylostoma duodenale* has the ability for arrested development in hosts whereby it can survive in the host and develop at a later time to continue the life cycle.[111] This possibly confers it with an evolutionary survival advantage. It has been proposed that increased climatic temperatures could facilitate survival and more optimal environmental conditions for the spread of endemic tropical STH infections into temperate non-endemic regions. Also there is concern of the increased risk to humans from zoonotic species such as *Ancylostoma caninum*. A report in 1995 proposed that the occurrence of autochthonous *Ancylostoma caninum* larva migrans was possibly due to increased excursions of the affected individuals to recreational activities in nature consequent to a higher than normal prolongation of sun exposure and higher mean temperature. [112]

2.7 Geo-helminth Infections in Children

Worldwide about 270 million children of the pre-school age children and about 600 million school age children live in endemic regions that have a heavy burden of STH diseases. The highest prevalence is seen in school aged children.[107, 113, 114] They are at risk and require therapeutic and annual preventive anti-helminthic chemotherapy.[115] An excellent review of the nutritional impact of Intestinal helminthiases during the human life cycle has been presented by Crompton and Nesheim.[116] The effect on the immune system due to the intestinal infection due to helminthes is reviewed by Koski and elaborates on how the parasitic infections affect the nutrition and cause micronutrient and macronutrient deficiencies that cause down regulation of the Th2 and up regulation of the Th1 immune response, which keeps the appetite of the affected individual low thereby perpetuating the pathologic process and protecting the worms form the Th2 immune response.[117]

2.8 Impact of STH Infection.

Helminthes impact human population in myriad different ways. Of our interest are the soil transmitted helminthes (STHs) or geohelminthes. Geohelminthes are mainly the nematode helminthes that have a developmental stage in soil. The eggs are shed in the feces are not infective as such. They need to spend some time in the soil in the environment that helps the development of the embryo and makes the eggs infectious to the next host although there are exceptions like the *Strongyloides spp*. and *Enterobius vermicularis*. They generally cause intestinal infection although *Ascaris spp*. can wander and end in unusual locations. *Strongyloides spp*. can end up anywhere in the body and has been known to cause meningitis in susceptible individuals.[118]

Children are particularly susceptible to geohelminth infections especially in the stage of their growth and development. They are still at a stage in life where they are still learning and forming life defining habits. Their play involves playing in the soil and they do not have the rigid habits formed yet regarding sanitation and hygiene. Some of them are yet to develop the sense of place propriety for bowel and bladder evacuation.

STHs are the poster-boys for helminthes and the most well-known of them is *Ascaris lumbricoides*. The other important members of this group that cause significant morbidity are *Trichuris trichiura* and the hookworms (*Necator americanus* and *Ancylostoma duodenale*). STHs mainly cause nutritional imbalances the different infections affect humans at different times of their life. Commonly, they cause a decreased appetite leading to reduced food intake, lethargy, impaired digestion and absorption of nutrients from the diet and thereby poor growth and development. This is particularly so with *Ascaris* and *Trichuris spp*.

Hookworms are primarily responsible for blood loss leading to iron deficiency and iron deficiency anemia. The hookworm disease can affect the developing fetus by causing anemia in the mother and also affects the working capacity of workers and early childhood development of infants and children. Iron being necessary for neurocognitive development, iron deficiency affects the neurocognitive development of children as has been suggested by various animal and human studies.[119]

Ascaris infection is present in about one quarter of the world's population. *Ascaris* infections have been called the 'forgotten disease of the forgotten people' since it was so widespread and common yet neglected.[120] *Ascaris* affects humans at all stages of their life. Ascariasis causes impaired childhood nutrition, surgical complications and allergic reactions. *Ascaris* worms express surface proteins for binding of retinol and retinoic acid and also enzymes for metabolism of retinol and so result in Vit. A deficiency of the host.[121] Suchdev et al. found an association with *Ascaris* infection and Vit A deficiency and iron deficiency in urban slums in Kenya amongst Pre-SAC and SAC. Their study also showed that co-infection with *Trichuris trichiura* to be associated with iron deficiency and iron deficiency anemia in those children.[122]

Trichuris trichiura is as common as *Ascaris* and lives in the colon and the rectum. The inflammatory changes associated with *Trichuris* have been known to cause rectal prolapse in individuals with heavy infestation. Aside from this dramatic effect, *Trichuris* has an effect on the appetite and nutrition of affected children by induction of cytokines known to suppress appetite like tumor necrosis factor – α (TNF-α). The appetite is decreased with reduced food intake and malnutrition from inadequate diet and competition. The nutritional deficiencies have been shown to cause reduced growth in children. Trichuris Dysentery Syndrome (TDS) is associated with intense and heavy *Trichuris trichiura* infection, and is characterized by chronic dysentery, rectal prolapse, anæmia, poor growth, and clubbing of the fingers.[122-124]

2.9 El Salvador: Overview of the Water Supply, Sanitation and Child Health.

2.9.1 Water Supply

The latest 2014 Joint Monitoring Programme of the WHO and UNICEF for Water Supply and Sanitation (JMP-WHO/UNICEF for WSS) estimates for El Salvador show that although a great increase in the access to piped water has occurred from 1980 to 2012, 27% of the country still lacks coverage by piped water, 10% still of the country still relies on unimproved water sources for their water needs. The piped water supply in El Salvador is through three mechanisms. One is through the 'Administracion Nacional de Acueductos y Alcantarillados' (ANDA), second is through the other is through the 'Operadores Descentralizados' (decentralized operators) and finally the 'otros operadores' or other operators. According to the 'Boletin Estadistico 2011' of ANDA, 3,938,495 people have access to potable water through piped connections provided by ANDA and the decentralized operators. The urban-rural difference in the coverage of piped water is quite striking in El Salvador. In the urban regions there is 86% coverage while in the rural parts, the coverage with piped water is only 49%. Also, while 5% of the dependence in the urban regions is on unimproved sources, in the rural regions it is 19%.[1, 101]

2.9.2 Sanitation

JMP-WHO/UNICEF for WSS shows that in El Salvador the coverage of improved sanitation facilities is only 70% and still nearly a quarter of the country still uses unimproved sanitation facilities with about 4% practicing open defecation. The urban-rural difference in the coverage of sanitation facilities is quite striking in El Salvador. In the urban regions there is 80% coverage while in the rural parts, the coverage with improved sanitation facilities is only 53%. Also, while 9% of the urban region still uses unimproved sanitation facilities, in the rural region sit is 42%.[1] According to the 2011 ANDA 'Boletin Estadistico', urban sanitation coverage by sewerage by ANDA for household was 66.1%. However, the report points out since 2007, ANDA and decentralized operators' systems do not have in their records with regards connections where feces disposal is through latrines. Furthermore, municipalities with sewers serviced by ANDA amounted to 64, representing 24.4% of all municipalities, while 8.0% (21 municipalities) are served through Operators Decentralized and for 67.6% (177 municipalities), no information is available.[101]

2.9.3 Child Health

Figure 4. Shows the current status of Acute Diarrhœal Diseases and Parasitic infections in El Salvador. It is evident that El Salvador still has a long way to go before the entire population has access to the safe drinking water source; similarly, for the provision of safe sanitary facilities (Figures 4 &5.). The current numbers of acute diarrhœal disease, gastroenteritis and intestinal parasitic infections indicate a potential for greater spread of helminthic diseases due to less than perfect safe sanitation and drinking water facilities in El Salvador. This coupled with the increasing population of El Salvador could translate into higher numbers of people affected (Figure 5.).

Indirectly from the above data it is evident that the persistence of STHs in El Salvador is likely as evidenced by the increasing cases of acute diarrhœal diseases and parasitic intestinal infections since the risk factors are the same.

The WHO indicators for STHs for El Salvador show that in 2013 there were 612,918 school age children (SAC) that required chemotherapy and of those 237,051 were Pre-school age children (Pre-SAC). The national coverage with chemotherapy was 31.56% for SAC.

El Salvador's progress towards the Millennium Development Goals (MDGs) for Sanitation, Safe water and child health

Three of the eight MDGs have targets concerning sanitation, safe water and child health. These are MDGs 4, 6 and 7.

2.9.3.1 MDG 4: Reduce child mortality by 2015

Target 4A aims to decrease the under-five mortality rate by two thirds from 1990 to 2015. Diarrheal diseases account for the second most common cause of death. [125] The 3rd report on the progress of the MDG issued by the Government of El Salvador states that by 2011, according to the Ministry of Health (MINSAL) data the under-five mortality rate had decreased to 9.3 which was more than the two-thirds decrease from the 59.5 in 1990 thereby accomplishing this MDG. [126]

2.9.3.2 MDG 6: Combat HIV/AIDS, Malaria, and other diseases by 2015

Target 6C of this MDG is to halve the incidence of malaria and other diseases by 2015. The other diseases include tuberculosis and neglected tropical diseases (NTDs). Amongst the NTDs are the STHs.[127] At the current time, there is no data pertaining to the STHs for the entire country of El Salvador.[126]

2.9.3.3 MDG 7: Ensure environmental sustainability by 2015

Target 7C of the MDG 7 aims to halve the proportion of people without sustainable access to safe drinking water and basic sanitation by 2015. According to WHO by the end of 2011 89% of the world's population used an improved drinking water source while 55% had a piped connection for supply of water. However, still 768 million people did not have an improved source for drinking water and a sixth of those depended on surface water sources for day to day to needs. Most of these people live in the rural regions. In Latin America and the Caribbean region, piped water supply increased from 72% to 89% while the dependence on surface water reduced from 7% to 2%.[128]

From 1990 to 2011 the percentage of people practicing open defecation decreased from 24 to 15%. However, there are still about 1.04 billion people that practice open defecation. The majority of these people reside in the rural areas. In Latin America and the Caribbean region, unimproved sanitation decreased from 10 to 8% and open defecation decreased from 17 to 4 %.[128]

The 3rd report on the progress of the MDG issued by the Government of El Salvador states that El Salvador has successfully accomplished the target 7C of MDG 7 based on 3 of the 5 of the indicators. However most of these pertain to safe drinking water. The proportion of population using an improved drinking water source (Form 1-well) increased from 63.3% in 1991 to 91.2% in 2012. This is more than the goal of 80.5% set for 2015. The proportion of population using an improved drinking water source (Form 2- without well) is at 84.2% in 2012. It does not have any baseline figures for 1991 and so the increase cannot be determined from that time-point. The proportion of population using an improved drinking water source (Residential connection and common source) increased from 42.2% in 1991 to 74.9% in 2012 whilst the target is 71.1% until 2015. The proportion of population using an improved drinking water source (private and common) increased from 76.7% in 1991 to 96.2% in 2012 whilst the target is 89% until 2015. When it comes to the sanitation indicator, the proportion of population using an improved sanitation facility (toilet to sewage pipe, septic well and private bathroom) is yet to be reached. It has increased from 72.9% in 1991 to 81.4 % in 2012. It still has to increase to 86.45% to reach the goal by 2015.

2.10 El Salvador: Rural Urban

The rural urban classification of places is important from the perspective of definition as well as resource allocation and analysis of disease trends. However, the definitions of rural and urban vary from country to country and therefore international comparisons are fraught with risk of ill-comparison. For El Salvador, the official policy of definition of ‘urban’ is ‘Administrative Centers of Municipalities’.[129] The remaining areas would be classified as rural. The sixth census of population of El Salvador of 2007 lists all the Departments and their municipalities’ populations gender-wise and into rural and urban. As the percentage varies for each municipality and department, there is no definition of when a municipality is considered as urban and when it is considered as rural. According to the 2011 report 60.3% population of El Salvador is considered as urban.[130]

The urban-rural difference in the coverage of sanitation facilities is quite striking in El Salvador. In the urban regions there is 80% coverage while in the rural parts, the coverage with improved sanitation facilities is only 53%. Also, while 9% of the urban region still uses unimproved sanitation facilities, in the rural region sit is 42%. There is also a significant urban-rural difference in the coverage of piped water in El Salvador. In the urban regions there is 86% coverage while in the rural parts, the coverage with piped water is only 49%. Also, while 5% of the dependence in the urban regions is on unimproved sources, in the rural regions it is 19%.[1]

Previous studies have shown that the burden of STH infections is higher in rural areas compared to urban areas. Also a study in China showed that accelerated economic development of a rural area and its transition into urban area accompanied by a transition from an agricultural to a commercial economy led to a significant decrease in the incidence and prevalence of STH infections. [131]

2.11 Ecological Zones in El Salvador

El Salvador is located in Central America on the Pacific coast. It has a tropical climate and have a varied ecological make up. It has varying terrain with varying surface water availability and use. Based on these characteristics, five different zones are identified. They are Coastal Range, the Coastal Plain, the Volcanic Chain, Central Depression (or central valleys) and Mountain Area that bordered to the north and are shared with Honduras.

The warm, moist climate is maintained throughout the year without much extreme variation nor extended and allows the biological development of the parasites in question. Differences in the micro-environmental factors of the geographical areas may also influence the distribution and prevalence of parasites in the territory. [132]

2.12 Sanitation and Soil Transmitted Helminth Studies in El Salvador

Historically, there have been studies in El Salvador that focus on sanitation and intestinal helminthes. In 1988, Reinthaler et al in their study looking at the stool samples of 210 children with diarrhea identified intestinal helminthes and protozoa in 49% of the children. Amongst the helminthes infections, 31% were *Trichuris trichiura*, 18% were *Ascaris lumbricoides* and 5 % were *Hymenolepis nana*.[133] In the early 1990s, the Ministry of Health of El Salvador along with UNICEF and USAID promoted the construction of thousands of ecologically sustainable latrines that were designed to transform infective human biowaste to non-infective soil conditioner by storage. Primarily two models were approved for construction. One was the 'Latrina Abonera Seca Familia' (LASF) which was a double vault desiccating latrine and the other was the solar desiccating latrine. These latrines were designed to operate by holding the human biosolid, ash and lime or sawdust additives for an extended holding time to inactivate the infective pathogens by an interplay of temperature, humidity, pH and time itself. However, the end user compliance with usage guidelines is not complete. According to the 1996 report on the evaluation of use and maintenance of latrines constructed by CREA International, El Salvador presented to USAID/El

Salvador Office of Health and Education, US$3.1 million was used for construction of latrines. The definitive evaluation of the use and maintenance of these latrines in 1995 revealed that at the household level 94% of the composting latrines were being used, 67% were being used and adequately maintained and 27% were being used but not adequately maintained. Microbiological testing of the samples taken from the latrines revealed that 39% were safe to be used as fertilizer, 25.5% had minimal tissues with total coliform counts and or fecal coliform with low pH and 35.5% of the compost had a high incidence of total and fecal coliform, with a neutral or slightly lower pH. None of the tested samples showed evidence of any *Ascaris* ova or any larvae.[134] In 2001 a survey of households in El Salvador looking at the sanitation behaviors and environmental conditions in desiccating latrines in El Salvador. Their total study population consisted of 2,587 persons from a total of 444 households with desiccating latrines in eight communities in El Salvador. Of these, DVUD were used by 89% of the households while the remaining 115 used solar latrines. Of these 90% had received some form of assistance from the either in the form of labor or material to build their desiccating latrine. More than 80% of the households reported receiving training in the use and maintenance of the desiccating latrines from either from an organization, health worker or a non-skilled person. All the households used some sort of desiccating material to their latrine vault, predominantly lime or ash. They found that 72% added the material after every use, 96% used two or more cups and 76% of the households had the material available inside the toilet cabin. However, only 19% of the respondents reported mixing the vault contents weekly, of which 17% were users of DVUD and 39% used solar latrines. On an average the household used the vault for about a year prior to sealing it. After sealing, 7% of the households opened it before the recommended six months storage time. While 93% of the DVUD latrine owners perceived that storage time should be at least 6 months to decrease the risk of contamination, 82% of solar latrine users perceived that storage time should be more than 30 days. Only 49% of the DVUD using households and 51% of solar latrine equipped households felt that the bio-material was safe for removal. Also, only 57% of the households stored the biosolids for over a year. On comparing the use of an additive and mixing practices amongst DVUD user in rural and urban areas, they found that rural user were less likely to use an additive and mix the additive. Also, rural users were more likely to use alkaline additives like ash, ash with lime or lime alone. Another finding was households that used free additive were less likely to mix it in compared to households that paid for the additives. In this survey, the pH of the bio-material was found to range from 5.07 to 12.72 with an average of 8.92 for the DVUD latrines, whilst it ranged from 6.79-12.76 with a mean of 9.84 for the solar latrines. Amongst the different additives used, they found that the highest pH was attained by the use of ash and lime combined together followed by ash alone and lime alone in that order. The average moisture content in the DVUD latrines was about 41% ranging from 1.8% to 98.3% while for solar toilets it was 33.6% with a range of 5.7% to 97.3%. For solar toilets the mean moisture content of the biosolids was greater if the use of additives was occasional and the storage time was less than 30 days.[135] Moe and Izurieta reported in 2003 on the longitudinal survival of fecal pathogens in biosolids from the double vault urine diverting toilets (DVUD) and solar latrines in El Salvador over a one year period.[136] They found that the most effective parameters for the inactivation of fecal pathogens were the peak temperature achieved in the storage vault and the pH. Also another important factor was the duration of storage with lesser duration times corresponding to lesser inactivation of fecal pathogens. They failed to find any viable *Ascaris* eggs in samples from the solar latrines where the peak temperature averaged at 37^0C and the highest recorded was 44^0C. Also, the toilets which had their solar panels facing south received consistently good sun exposure had higher peak temperatures in the vault. This coupled with higher lime mixing by the users resulted in higher pH of the biowaste at around 10.3 and better inactivation of fecal pathogens.[136] The 2004 report on the operation and safety of dry latrines in El Salvador by the Ministerio de Salud Publico Y Assistencia Social reported that 96.03% of adults 18 years, 91.72% of children aged 6-18 years, 37.39% of children aged 3-6 years and 11.32% of children under 3 years of age used latrines.[137] Some of its significant recommendations include the promotion of construction of the Type III prototype of solar latrines in such a way as to ensure that the holding chamber possessed sufficient capacity to hold the biosolids and get adequate exposure to sun light. Also, the storage time for the LASF latrine should be 1 year while it should be 8 weeks for solar latrines that are operated per standard operating guidelines. The use of ash with lime or at least one of the two additives was recommended, while the use of sawdust was discouraged due to the low pH levels observed with sawdust use. They also recommended the installation of air pipes to ensure adequate desiccation of the biowaste in the holding chambers. They advised caution in the use of the biosolids for use in agriculture and recommend that consumption of fresh vegetable and fruits produce that could possibly be contaminated with it, particularly the produce from creeping vines. It also recommended that the children's education program include training on the use of the latrines and the importance of maintaining the environment. The capacity building activities in the use of latrines are a continuous process and should include a packet for education. Facilitate the families using ecosanitation to gain access to ash and lime or help them obtain them. Further recommendations were made to adjust the size of the holding chamber based on the size of the family using the LASF and to even build an extra chamber there were extended family using the LASF.[137] In 2006, Corrales et al found that in their study sample of 107 households, LASF was used by 31 households, solar trines by 20, pit latrines by 31 and 20 households had no latrines.[138] Dry latrine use was associated with lowest prevalence of STH infections. Amongst these the solar latrines were associated with the lowest prevalence numbers for enteric parasitoses. *Ascaris lumbricoides* and *Trichuris trichiura* were not completely inactivated by use of double vault urine diverting toilets although they were associated with reduction in transmission of some fecal pathogens. They found that 53% of their study population had intestinal parasitic infections. Of those *Ascaris lumbricoides* accounted for 8%, *Trichuris trichiura* for 22.9% and hookworms for 20.3%. Children in the age-group 6-12 years had 1.5 times higher risk for infection with *Ascaris* when compared to other age groups and they also had the highest percentage of infection with *Trichuris trichiura* when compared to all age groups. Adults had the highest infection rates for hookworms. Also, they found that the prevalence of hookworms was highest in households that owned pigs and households with dirt floors were associated with higher prevalence for *Trichuris trichiura*. Males had a higher prevalence for infections with *Ascaris*, *Trichuris* or hookworms when compared to females and agricultural occupations were associated with higher hookworm infections. On testing the association between the type of sanitation and STH infection they found that LASF appeared to be protective against hookworms but was associated with the highest prevalence of *Ascaris* and *Trichuris* infections. Pit latrines were found to be protective against *Trichuris* but offered no protection against *Ascaris* or hookworm infection. They also found that, if the human biowaste was kept buried in the pits in pit latrines, the household members were less infected with *Ascaris* and *Trichuris* compared to household member with eco-san latrines in which the biowaste was buried in the yard after storage in the holding chamber. They hypothesized that the transmission of STH was more likely to occur during the removal of the biowaste from the latrine and after contact with the buried biowaste. Particularly, the risk for *Ascaris* infection was 8.3 times and for *Trichuris* infection it was 3.7 times.[138] Colston and Saboya using geospatial modelling in 2013 estimated that the number of SAC that are estimated to have STH infection was 439,389 out of 1,218,719 SAC giving an estimated prevalence of 36.1% for SAC necessitating 273 municipal level of rounds of MDA annually.[139]

2.13 Toilets Used in El Salvador

There are many different types of toilet designs currently in use in El Salvador. The main designs of the latrines in use in El Salvador are the pour and flush systems, composting latrines, solar latrines, pit latrines and open air latrines. The availability of water per person dictates the type of toilet that is constructed.

2.13.1 Water Pour Flush Toilets

Availability of greater than 25 lpcd (liters per capita per day) of water is necessary to construct the water pour flush systems. When the water availability of the water is greater than 60lpcd then the water pour flush systems can be connected to a sewerage system to drain the flushed biowaste. The design of these toilets involves a receptacle of porcelain either in the form of a bowl or a flat pan with a seat over the bowl or pan or a raised platform to squat over the pan. The flush user uses the water to cleanse and then pours water into the toilet to flush the biowaste deposits. Flush can also be by activating flush tanks that hold water. These flush systems may have two different levels of flush water quantity by employing ½ gallon for flushing liquid wastes and 1 gal flush for flushing solid wastes. The toilets are inodorous due to a water seal which ranges from 25 to 50mm. They are very popular and very easy to clean. In these types of toilets there is no urine diversion as both the liquid and solid biowaste are flushed with water. These toilets can be connected to a septic tank or main sewerage systems for transport of the biowaste to a central treatment plant.[140]

2.13.2 Composting Latrines.

This is a type of toilet that is designed for use in regions where the availability of water is less than 25lpcd. The design is based on the concept of sanitizing the human biowaste by storage for prolonged periods of time. The structure is an elevated privy chamber with commode or hole in the floor into which the feces is deposited. It sits atop a collection cum holding chamber which is usually at a ground level. Usually, there is a bi-cameral holding chamber such that when one is full, the other is used and first is closed for prolonged storage of the accumulated human biowaste. Prolonged storage of feces results in inactivation of fecal pathogens due to natural biological processes. However, studies have shown that the inactivation of fecal pathogens in such latrines is dependent on the storage time and the temperature achieved in the pile of human waste. The temperature inside a composting latrine is generally only 10C greater than the ambient temperature. Additionally, moisture and pH play a vital role in the inactivation process. The main inspiration for the promotion of this type of latrines was to generate recycled human fecal matter as a soil conditioning fertilizer.

The best use recommendations for the latrines suggest have two or more chamber depending on the number of family members using the latrine and having chambers of adequate capacity so as to ensure adequate duration of storage of the fecal material for inactivation of fecal pathogens. Also, urine diversion designs are promoted to enhance the acceptance and also the user experience, the urine is separately collected in a container and then emptied into a pit in the ground for percolation.

The disadvantages of the composting latrines are mainly based on its design and operation. It takes a long time for the human biowaste to be safe for use as fertilizer, the chamber need to be periodically emptied, If the chamber is full, you cannot use it , so must have adequate sized chambers, they can be smelly if there is no urine diversion. Based on the evolution of the composting latrines, the currently used designs are the double vault urine diversion (DVUD) systems that have at least two vaults for collecting and holding the feces and a receptacle for collection and diversion of the urine separate from the feces. Also, the users are recommended to use ash and or lime as additives after defecation to minimize the odors and mix it with the feces. This helps improve the efficiency of the composting latrine in inactivation of the fecal pathogens.

2.13.3 Solar Latrines

The understanding of the parameters for the inactivation of the fecal pathogens led to the designs of latrines that retain the chambers for collecting and holding of feces like to composting latrine, but additionally now have the holding chamber that is jutting out from the base of the toilet structure. The chamber has a metal lid painted black that faces southwards in El Salvador to ensure maximum exposure to the radiant energy of the sun. The lid is called the solar panel. This black solar panel helps trap the radiant heat of the sun. Consequently the temperature inside the chamber rises much higher than the ambient temperature often reaching peak temperatures of above 44^{0}C. The operation of the solar latrine is otherwise similar to the composting latrine. The notable differences are that the solar latrine needs shorter storage times for the sanitization of the human biowaste into a safe soil conditioner for use in the fields. Also, the designs of the holding chamber makes it easier for the user/operator of the toilet to move the biowaste from one chamber area to another. This sanitation technology developed in El Salvador has already met two important sustainability aspects, socio-cultural and institutional acceptance, and has proven to reduce enteric diseases among communities using this technology.[136] The challenges with the solar toilet are that the toilets are best designed for regions with plentiful sunshine all through the year to ensure adequate heat capture and inactivation of fecal pathogens. Having trees or houses cast a shadow on the solar panel adversely influence the efficacy of the solar toilet in inactivation of the fecal pathogens. As with the composting latrines, users of the solar toilets also use ash and or lime and mix it with the feces to eliminate odor.

2.13.4 Pit Latrines

These are probably the simplest of the designs and at the very least consists of a pit in the ground that is used as a receptacle for the deposition of human biowaste with a shelter to ensure privacy. The more refined designs have a ventilation pipe to minimize the odor. These are well suited for places where the water table is deep and regions not prone to flooding. Once the pit is full, it is covered and if there is plenty of ground available, another is dug. If ground is not available, it is covered with a concrete or plastic seal and kept so for over a year and another pit is used. The two pits are used in rotation, emptying them at the end of the storage periods. These tend to attract flies due to the smell and be less popular. If very poorly maintained, individuals opt for open defecation to avoid the unpleasantness of the foul smell and unclean toilet.[141]

2.14 Inactivation of Fecally Transmitted Soil Transmitted Helminthes

Fecally transmitted pathogens are primarily transmitted through oral ingestion except for hookworms Strongyloides larvae which can penetrate skin to cause infection. The digested human biowaste is rich in nitrogen and thus an excellent fertilizer for use in the farms and fields. However, when used without treatment, it poses the risk of contamination of the foods and food sources. Human biowaste is cheap, readily available and this cost saving for the poor farmers in the developing world. Numerous different types of unconventional localized sanitation technologies have been tried in non-urban areas in the third world countries to meet MDGs.[142] Focused on sustainability issues such as minimal requirement use of water for operation. However, they fail to meet the standards of safe disposal of the human biowaste due to inadequate inactivation of the fecal pathogens particularly, the ova of STH.[143] Numerous researchers have evaluated the factors affecting the inactivation in the Ecosan technology designs and determined that the critical determinants for inactivation of STH infective forms are peak temperature, duration of treatment, pH and ammonia concentration in the biosolid. *Ascaris lumbricoides* has been found to me most hardy fecal pathogen and so is used as the indicator organism in studies for the efficacy of techniques and technologies for inactivation of fecal pathogens.[144] The interplay of temperature, pH, and concentration dissolved ammonia and the duration of treatment determines the inactivation of the STH ova.[145] Pecson and Nelson in 2005 demonstrated the efficacy of ammonia in the inactivation of *Ascaris suum* eggs in the laboratory.[146] Further studies by Nordin et al elucidated the efficacy of ammonia on inactivation of *Ascaris* eggs in source separated urine and feces at ambient temperatures.[147] Wastewater and sludge water treatment are not 100% effective in the destruction of *Ascaris* eggs. The eggs survive many years in the environment despite harsh conditions.[148, 149] The hardiness of the *Ascaris* eggs have earned them the status of indicator for quality of hygiene.[149] Ammonia has been studied for inactivation of *Ascaris* as early as the 1970s. *Ascaris suum* eggs in solid and biological wastes were reported to be inactivated between 18-22^{0}C in under 2 weeks.[150] Subsequently, in the 1990s, researchers have focused on the use of ammonia in solution for inactivation of *Ascaris* in sludge.[147, 151, 152] Urea is an attractive additive to feces for inactivation of fecal pathogens particularly the STH ova because it offers the optimal required physico-chemical conditions at a very low cost.[153, 154] Also, the use in particularly effective in dry toilet technology designs as it helps sustain the low demand of precious water resources and need for expensive complex sewerage systems while according health protection to the users and recycled fertilizer for agriculture. Urea, aside from being inexpensive, is a good source of nitrogen (46%) and so is widely used as a fertilizer in agriculture. Urease enzyme in soil breaks down urea into ammonia and bicarbonate ions but a significant amount is lost to the environment due to the volatile nature of ammonia.[155, 156] Factors that affect the urea breakdown volatilization of ammonia are temperature, other chemicals, urea concentration and pH.[156-158] Some studies have reported on the effect of ammonia fumes on the inactivation of *Ascaris* ova. [159] While the exact mechanism of action of ammonia on inactivation of *Ascaris* ova is unknown, it has been hypothesized that higher temperatures enhance the lipid membrane permeability of egg shell that facilitates the entry of unionized ammonia and disruption of intracellular cytoarchitecture due to increased pH leading to inactivation.[146, 154] Ova of other STH species such as *Trichuris spp*, *Hymenolepis* and *Diphyllobothrium latum* have also been shown to be susceptible for inactivation by ammonia.[160-162]

Heat is a potent agent for inactivation of *Ascaris* ova, however, the temperature required is greater than that reached inside the toilet chambers that are in use in communities.[163, 164] Factors that preclude the attainment of high temperatures intra-camerally in the community toilets include, shade of tress, shadow of the house, non-orientation of solar panel to capture greatest amount of sunlight, breaches of integrity of the solar panels themselves comprising effects of a closed chamber, use of stones and rags by users to avoid stench. Studies by Pecson et al and Nordin et al have demonstrated that addition of urea, increases the ammonia concentration in the biosolid and lowers the level of temperature and duration of treatment required for inactivation of *Ascaris* ova in fecal matter.[147, 152] Ghiglietti et al did not find an association between the pH level and *Ascaris* inactivation and Nordin et al surmised that while urea addition increases the pH of feces, it is ill sustained due to volatile losses of ammonia.[151, 154]

Studies have shown that long storage time for 2-3 years may be necessary depending on the conditions of temperature and moisture in the fecal mass during storage for inactivation of fecal pathogens. Also, higher temperature and lower moisture levels help accelerate the inactivation process.[149, 163, 165, 166]

In 2010, Ligia Cruz simulated the physico-chemical conditions in the holding chamber of solar toilets and tested the viability of *Ascaris suum* ova. In that study ammonia was effective in inactivating *Ascaris suum* ova provided certain physico-chemical parameters for storage of fecal matter were met. That included having a closed chamber with temperature minima of 28^0C, pH of 8.3 and urea additive at 1%w/w in feces. There was an inverse relation between the temperature and the duration of storage required for 100% inactivation of *Ascaris suum* ova.[153] Moe et al have reported high temperature coupled with high pH to be the single most important determinant for inactivation of *Ascaris* while Yang et al reported that higher pH was associated with higher inactivation rates while higher moisture content was associated with lower inactivation rates for *Ascaris suum* in solar toilets.[163, 166] However, there are no studies that have reported the efficacy of urea as an additive for inactivation of *Ascaris* ova when used in the solar toilets in community settings.

2.15 Integrated Control of Soil Transmitted Helminths

Integrated control of soil transmitted helminthes (ICSTH) concept has been around for over a 100 years. The Rockefeller Sanitary Foundation, created in 1910, employed a three pronged approach for the control of hookworm diseases in the Southern United States. The focus was on prevalence mapping, treatment of infected individuals and public health education for promotion of improved sanitary habit to decrease the practice of open defecation. Notably, the program laid great emphasis on public health education by explaining the parasite lifecycle and importance of improved sanitation.[167] Previous ICSTH programs have included improvement of sanitation combined with mass drug administration as reviewed by Schad, who also noted an absence of public health education programs for control of STH infections.[168]

A similar sounding but completely different concept is the integrated control of Neglected Tropical Diseases (ICNTD). It is sometimes referred to as Integrated Neglected Tropical Disease Control (INTDC). While ICSTH primarily focusses all attention to the different mechanisms of interruption of transmission of STH infection that include treatment of infected individuals, safe sanitation, public health education and co-ordination of various agencies both public and private for this mission, INTDC focusses on the control of STH as part of a larger program of control of other NTDs like malaria, dracunculiasis. While the intentions are appreciable, in the sense of maximizing the utilization of scant resources available in developing countries, it does end up being focused only on one aspect of the complete spectrum of interventions essential for the control of STH infections. Also, frequency of interventions and the scale of implementation are different leading to a final effort that is un-coordinated from an ICSTH perspective.[169, 170]

Based on the current understanding of the biology of STH infections an ICSTH approach could broadly be summarized as follows:

- Source Reduction: Treatment of hosts (human and non-human) and Vaccination

- Environmental management
- Behavior modification through public health education
- Other Supportive Activities

2.15.1 Source Reduction

2.15.1.1 Treatment of host – mass drug administration

This is done therapeutically as well as prophylactically. Therapeutically, an infected individual is treated with a dose of antihelminthic medicine once. The commonest drugs used are mebendazole and albendazole.[171] Other candidate drugs are pyrantel pamoate and levamisole.[172] Albendazole is popular as it is safe for use in children and adults and only a single dose is required.[173] In prophylactic treatment which is also known as preventive chemotherapy (PCT), the antihelminthic medicine is administered to uninfected at risk individuals periodically. Again, the drug used is either mebendazole or albendazole.[174] Since the year 2010, GlaxoSmithKline (GSK) has donated 400 million albendazole doses annually free of cost for the MDA program for PCT for control of STH infections. GSK commitment is until 2020. Additionally, Johnson and Johnson has committed 200 million doses of mebendazole for donation to WHO for PCT program for STH.[175] Typically, the drug is administered at least once or twice a year depending on the prevalence of STH infection. WHO, recommends once a year PCT for STH infections in communities with greater than 20% prevalence and twice a year if it is greater than 50%.[176-178] However, different schedules have been used by different countries and as many as 6 doses have also been administered in a year. Independent studies have demonstrated the beneficial role of mass drug administration (MDA) of anti-helminthic medications in reducing the prevalence of STH infections.[179] Studies have shown that the age-group most heavily infected in the general population is the school age children (SAC) and pre-school age children (pre-SAC).[180, 181] Hookworm burden is higher in adults. Accordingly MDA strategies target, SAC, pre-SAC, women of child bearing age including women in second and third trimester and, adults in certain high-risk occupations, such as tea-pickers or miners .[177] In regions where hookworms are the predominant STH infections MDA is administered to adults as well.

MDA had been considered by WHO as a strategy for control of STH infections as early as 1961. However, the then extant drugs were not as efficacious as current drugs.[182] According to WHO, 106 countries are in need of preventive chemotherapy for STH infections. Of those only 55 countries report PCT for pre-SAC and 61 countries report PCT for SAC.[183] However, Anderson et.al. determined that for MDA to be successful in elimination in transmission of soil transmitted helminthes, it must include adults along with SAC and pre-SAC.[184] Also, another concern is the lower efficacy of albendazole against *Trichuris trichiura* necessitating multiple doses, which are typically not done in preventive chemotherapy cycles.[185] Drug resistance to benzimidazole compounds like albendazole has been considered to be responsible for the lower efficacy of albendazole in lowering the prevalence of STH infection in Kintampo, Ghana.[186] However, a counter argument is that the lowered efficacy reported may not be due to emergence of drug resistance but poor study designs, lack of standardized protocols and diagnostic techniques amongst others.[187] MDA is the most widely implemented strategy for the control of STH infections. Many studies have repeatedly determined the benefit of MDA in improving the growth and children of children in endemic regions.

2.15.1.2 Treatment of domestic and peridomestic animals

In regions where there is a high prevalence of zoonotic hookworms, human infections with these zoonotic hookworms has been documented. The clinical picture in humans due to zoonotic hookworm infection is similar to the human hookworm infections. Inpankaew et al in their study in Cambodia determined very high infection rates of dogs with canid hookworms by molecular techniques (95.7%) compared to microscopic techniques (80.9%).[188] In humans, their study found the numbers to 26.6% by microscopy versus 57.4% by PCR techniques. They found high (51.6%) but equal level infection with *Ancylostoma ceylanicum* and *Necator americanus* in humans while infection with *Ancylostoma duodenale* was low (3.2%). They also identified one dog that was shedding eggs of *Necator americanus* which is a known human hookworm.[188] Memon et al in their 2014 study identified that hookworm was the most common intestinal parasite in dogs of pet owners in the community in Karachi with higher prevalence of enteric in parasitoses in male dogs compared to female dogs and in puppies compared to adult dogs.[189] They recommend regular treatment of pet dogs to minimize the risk of transmission of zoonotic STH from infected dogs to humans. Schär et al in their 2014 study identified hookworms as the most common intestinal parasitic infection among dogs in Cambodia.[190] The dogs were also hosts for *Trichuris spp.* They found pigs to be hosts for *Ascaris spp*. and *Trichuris spp* of STH. Studies have demonstrated low level of awareness of pet owners of the risk and modes of transmission of zoonotic STH infections to themselves.[189, 191] Traub in a 2013 review article stated that human infections with *Ancylostoma ceylanicum* have now been reported in all geographical regions. Also, despite being a zoonotic infection it can have completely anthroponotic cycles thereby highlighting the necessity for the 'One Health' approach for control of STH infections that target both animals and humans.[192]

2.15.1.3 Vaccination

As the years of data and experience with the other interventions for the control of STH infections accumulated, the search of a cheap and viable option continues. Vaccination against the STH is an exciting that has been under research for a few decades now. The earliest work has been done on the development of a vaccine targeting hookworm infection in dogs in the 1970s against *Ancylostoma caninum*. It was a successful intervention using radiation attenuated infective stages of the larvae that induced protective immunity, but the high cost of production and distribution led to its discontinuation.[193] Hotez et al working with the Sabin Vaccine Institute Product Development Partnership were granted a patent on a vaccine against hookworm that had an antigen targeting the L3 larval stage (Na-ASP2) which is a protein secreted by the larva upon subcutaneous entry into the host and another antigen (Na-GST-1) targeted against the adult stages coupled with an adjuvant. This vaccine is currently under Phase I trials.[194] An ideal hookworm target product should conform to a profile that can be storage between 2^0C-8^0C, have 80% efficacy in reducing worm burdens, need intra-muscular administration, be administrable to children under 10 years of age, need maybe 1-2 doses and be cheap. Currently a Phase 2-b proof of concept trial is being planned for the future to demonstrate efficacy in preventing infection and reducing blood loss in Brazil, technology transfer to FIOCRUZ/Bio-Manguinhos for vaccine production for phases 2 and 3, process of registering the vaccine in Brazil for regulatory approval and simultaneously applying to WHO for pre-qualification and demand forecasting to determine the continued demand for the vaccine in endemic regions for commercial viability and sustainability.[193, 195, 196]

In 2009, Matsumoto et al reported the efficacy of a chimeric protein from *Ascaris suum* (As16) when coupled with cholera toxin B subunit (CTB) along with mucosal cholera toxin (CT) adjuvant in reducing the number of larvae in the lungs after challenge with embryonated eggs in pigs. This is an exciting and attractive vaccine candidate since the human round worm *Ascaris lumbricoides* also has a protein identical to the As16. Both the adults and larval stages expressed the protein in intestine, hypodermis and cuticle. This vaccine is produced in transgenic rice endosperm and accumulates in rice seeds to reach a concentration of 50μg a of rice seeds and can be administered orally.[197, 198] The Sabin Vaccine Institute Product Development Partnership is currently developing a five year plan to develop a 'Pan-helminthic Vaccine' that would be effective against hookworms, *Ascaris* and *Trichuris* infections. It plans to build this vaccine by incorporating the two hookworm antigens and one or two antigens from *Ascaris* and a new antigen identified from *Trichuris* called the stichosome antigen.[199]

2.15.2 Environmental Management

2.15.2.1 Safe sanitation

STH ova are expelled in feces. When the feces is indiscriminately disposed in such a manner as to come in contact with soil, the ova find ambient environment to further develop and mature into infective forms. Numerous approaches have been tried and are being used to process human feces to eliminate infective pathogens prior to disposal into the environment. These include holding, heating and desiccation, burial, composting, chemical treatment and combinations thereof. Based on the method of treatment, toilet designs have emerged that facilitate one or more feces treatment techniques aiming for safer sanitation. Zeigelbauer et al in their 2012 systematic review of effect of sanitation on the STH infections determined that sanitation was associated with a decreased risk of transmission of STH infection to humans and suggested access to sanitation should be prioritized to decrease the global burden of STH infections along with MDA and health education. [200]

2.15.2.2 Treatment of contaminated soil

This is necessary to eliminate the soil reservoir of infective ova. Multiple studies have identified infective ova and larvae in soil. A study in Titagarh, India found that 68.6% of samples from wastewater irrigated soil were positive for STH infective forms that included embryonated ova of *Ascaris lumbricoides*, *Trichuris trichiura* and hookworms.[201] In the veterinary sciences, treatment of soil for elimination of hookworm (*Ancylostoma caninum*) ova/larvae has been tried. The agents that have been tested include methyl bromide, sodium borate, urea, calcium cyanamide, dichlorpropene, and ethylene dibromide. However, methyl bromide is toxic to humans and calcium cyanamide is lethal to dogs. [202] Condensed tannins have been shown to inactivate the larvae of hookworm pathogens of canids and felids such as *Ancylostoma caninum* and *Ancylostoma ceylanicum* that can also infect humans. They have recently been shown to impair the motility of L3 larvae of *Ascaris suum* in vitro.[203]. Recent studies in Brazil have shown the activity of fungal extracts to be ovicidal for STH. Hofsttater has demonstrated the ovicidal activity of fungal extracts on the ova and larvae of *Ancylostoma spp*.[204] Also, Ferreira demonstrated the robustness of in vitro ovicidal activity against *Ascaris suum* ova of *P. chlamydosporium* fungus after passage through pig gastrointestinal tract, making it a viable option for treatment of soil. Similarly, the activity of *Duddingtonia flagrans* against *Oesophagostomum* species which is a cryptic STH of public health importance causing infection of humans, non-human primates such as red colobus, chimpanzees, olive baboons and l'hoest monkeys, ruminants and pigs in countries such as Uganda, Togo and Ghana where humans live in close proximity to these animals.[205-208]

2.15.2.3 Treatment of water source

The treatment of water source is generally thought of as an intervention for prevention of other fecal transmitted diseases such as bacterial, viral and protozoal infections. The fecal contamination of water can occur at source, during supply or during consumption. The contamination at source such as in wells, rivers, ponds and lakes can occur due to the practice of open defecation and anal washing. Infective forms of STH ova like *Ascaris* have been known to survive in fresh water bodies.[209] Udonsi described a laboratory and field based study on the detection of hookworm eggs and their viability in the Niger delta of Nigeria. Viable hookworm eggs were recovered from both fresh water and brackish water and the eggs from both these sources upon culture in the laboratory developed into infective L3 larvae. Also, viable L3 larvae were recovered from both fresh water and brackish water.[210] Many a times, the water supply and sewage drainage pipes run parallel. Water thieves breach the integrity of the supply pipes. This, in times of low pressure, can suck in the surrounding water and soil that is contaminated with fecal matter. Lastly, the hygiene habits of the consumer or the server can lead to contamination at the time of consumption of clean and pure supplied water. So water treatment should be at source and also at the point of consumption by use of filters or other water treatment units that use UV radiation or reverse osmosis.

2.15.3 Behavior Modification Through Public Health Education

2.15.3.1 Behavior change and water and sanitation.

Human behavior is the tie that binds disease-host-environment together in the epidemiological triad of disease causation. Therefore, intuitively, the first and most important intervention for disease control is to change the behavior that facilitates the agent-host and environment interaction leading to disease causation. Whitby et al in their 2007 review article summarize the different models and theories to explain human behavior as being focused on intra-personal factors like knowledge, attitudes, beliefs and personality traits; interpersonal factors like family, friends and peers and lastly the community.[211] Whiteford and Vindrola-Padros, in their recent book ‘Community Participatory Involvement: A Sustainable Model for

Global Public Health', summarized behavior change models into four categories- motivational models, behavioral enaction model, multi-stage model and social/environmental models.[212] The first category is called the motivational model in which there is an inherent motivation for an individual to engage in a particular behavior. From a public health stand point, enhancing the motivation for positive benefit behaviors leads to success of behavior change intervention public health programs. The motivational models can be of further three types – health belief model, protective motivation model, and theory of planned behavior. Health behavior model explains individual behavior by looking at the perceived individual vulnerability to the intensity of risk factor and the relative positive or adverse effects due to the risk factor along with any cues to engaging in positive health behavior. According to the protection motivation theory, an individual's decision to engage in a beneficial or harmful health behavior is dependent on the perceived risk of adverse event that threatens his/her health. The theory of planned behavior assesses an individual's decision to engage in health protective behavior through the lens of his/her motivation for such an engagement and their perceived control of circumstances to do so. The second group of models is behavior enaction model which includes information-motivation-behavior skills model. The information motivation behavioral skills model posits that the practice of positive health behavior by an individual is an outcome of the available information regarding the behavior, the social support and individual attitudes, and the skills necessary for practicing that behavior. The third type of model is the multistage model which includes the trans-theoretical model and finally the social/environmental model which includes the social ecological models. The transtheoretical model looks at the different stages in the behavior change process. It begins with the pre-contemplation stage when an individual does not intend to change behavior followed by the contemplative stage in which the individual thinks about the behavior change. This is followed by preparatory stage in which the individual gets ready to change behavior followed by action phase in which the behavior change is brought into effect. The last stage is the maintenance stage in which the behavior change is sustained. Finally, social ecological model tries to explain an individual's behavior based on the complex interaction and interdependence of the social determinants, individual qualities, community and institutional factors in light of the policy determinants at play in the individual's sphere of activity. A deeper understanding theoretical framework of these models is necessary in the planning of behavior change interventions for public health.

Guinea worm disease which is transmitted via the consumption water harboring copepods is now eradicated simply through the promotion and adoption of a behavior change that was as simple as use of filtered water for drinking. The use of cloth filter to filter water and use of filter-pipes for drinking water from untreated surface water sources is one of the behavioral interventions that has been successful in the control and eradication of guinea worm disease.[213] Possibly inspired from this, a study in Bangladesh found that use of folded sari for filtering water was also successful in reducing the contamination of water with diseases causing cholera organisms. While the sari cloth does not filter out bacteria, cholera organisms are known to inhabit aquatic environments attached to the chitinous exoskeletons of aquatic micro-fauna. The sari fold traps these and along with them the attached cholera organisms.[214] These interventions emphasize the importance of understanding the social customs and respecting them for a successful adoption of behavior change in prevention water-borne diseases. Saris are the daily wear of the women-folk in the South Asian subcontinent and easily available to all without any additional expense. Being so, they are part of the socio-cultural fabric and inherently accepted by the community at which the behavior change intervention is targeted. This is an excellent example of sustainable low cost behavioral intervention for control of water-borne illness.[215] All the models described above except the transtheoretical model can explain the behavior change of using a sari filter for water filtration in Bangladesh. For the guinea-worm eradication program all the models are capable of explaining the behavior change.

Understanding the social customs and beliefs that influence human behavior are of prime importance for success of any community based public health program. This is particularly so in the field of public health. The most significant model for planning a behavior change of a community is the social ecological model. As it takes into account the knowledge, attitudes and skills of an individual along with the formal and informal social network factors like friends, family, community groups, social institutions, organizational and institutional inter-relationships and the local and national laws and regulations. Community Participatory Involvement (CPI) involves the implementation of behavioral change interventions through participation of community stakeholders based on the principles of equity and equality engendering leadership.[216] Using the social-ecological model and CPI for planning and implementation of behavior change interventions in communities is successful.

During the cholera epidemic in Ecuador in 1991, numerous behavior change interventions were implemented to control its intensity and spread. These included campaigns to promote handwashing with soap and water, avoidance of eating ceviche which is prepared from raw sea food and can harbor vibrio cholerae organisms. Water hygiene was promoted by recommendations to use sodium hypochlorite for purification of water to rid it of vibrio cholerae. Also, use of a dedicated ladle to pour water from container was advocated to avoid contamination of the entire store of drinking water. The last rites customs of the indigenous population in Ecuador included the holding of body the deceased in the house for visitation by relatives to offer their last respects. At these events, the other household members were responsible for providing food for the visitors. During the epidemic, this led to an increase in the number of cholera cases and spread of the epidemic. Post-mortem the relaxation of the anal muscles caused release of the watery diarrhea and contamination of the clothes used to wrap the body. The household members responsible for the cleaning of the deceased were usually also the ones tasked with making food for the visitors. Contamination of the food prepared in the household by cholera organisms thus occurred and the epidemic spread. The identification of this custom led to the implementation of a recommendation of use of impervious body bags to hold the deceased. Also, instead of the five day holding period, it was recommended that those deceased due to cholera should be buried within 24 hours and feasting the homes of the deceased be not practiced. This resulted in the arrest of transmission of cholera from these events.[217, 218] From the social-ecological model perspective the individual factors such as the attitudes, skills and knowledge of individuals along with the factors such as family, peer, community in presence of organizational and institutional factors and regional and national laws were useful in planning the implementation and CPI was instrumental in successful implementation of the behavioral interventions in Ecuador during the cholera epidemic.

2.15.3.2 Handwashing behavior

Handwashing is an important behavior that has been shown to be effective in reducing the number of nosocomial infections, infections in children and in reducing school absenteeism among children.[219-222] Jumaa in his 2005 review identified that while the act of handwashing is a simple act it has a complex socio-behavioral aspect when it comes to its practice.[223] In a 2006 research article, Whitby et al describe that the handwashing behavior is of two patterns. First is the inherent one that occurs when hands look or feel dirty and the other is the elective handwashing in which the hands are neither look nor feel dirty. This is the behavior that needs to be reinforced in children for preventing transmission of STHI. Also, the inherent handwashing behavior patterns generally become established ere the end of first decade of life beginning with toilet training of the child.[224] Curtale et al in their 1998 paper recommended that health education for prevention of soil transmitted helminth infection should focus on promotion of positive health behaviors such as handwashing rather than knowledge of the diseases particularly in illiterate population using mass media such as televisions.[225] Freeman et al in their 2013 cluster randomized trial that evaluated the impact of school based hygiene, water quality and sanitation intervention on soil transmitted helminth re-infection conducted in forty government primary schools in Nyanza, Kenya found that the intervention reduced the intensity of *Ascaris* reinfection in school children but did not find any effect on re-infection with *Trichuris trichiura* and hookworm.[226] The interventions included hygiene promotion, handwashing and drinking water storage containers with 1 year supply of point of use water treatment product (‘Waterguard’) and latrines.

2.15.3.3 Cleaning food exposed to potentially contaminated soil

Food grown on farms is at high risk of contamination with STH ova, when human biowaste or sewage is used to fertilize or irrigate the farms.[227] Use of human and animal biowaste or sewage for growing vegetables is a common practice. If crops grown in such fields and farms is consumed without adequate washing or cooking, the consumer can get STH infection.[228, 229] There have been outbreaks of Ascariasis associated with use of sewage and human waste for as fertilizer.[230] The common vegetables and fruits that have found to be carrying helminth eggs include parsley, lettuce, strawberries, cabbage, garden-egg, carrot, cucumber and spinach.[228, 231] As much as 50% of the vegetables sold in the market in a study in Iran were found to be contaminated with infective forms of parasites. Vegetables bearing broad leaves like spinach are more likely to be contaminated with parasites.[227, 232] Gupta et al found that 44.2% vegetable samples in their study that were grown in wastewater irrigated soil were positive for embryonated eggs of *Ascaris lumbricoides*(36%), *Trichuris trichiura*(1.7%) and hookworms(6.4%). They identified the most contaminated vegetable to be Pudina (mint) followed by lettuce, spinach, coriander, celery and parsley.[201] Vegetables and fruits are consumed raw for their flavor, texture and nutritional benefits and are an important part of a healthy diet including the vegetarian and vegan diets. Consumption of such contaminated farm produce in the raw form exposes the consumer to the risk of infection with STH.[227, 228, 232, 233] The raw vegetables must be properly washed and disinfected before consumption to prevent transmission of STH infections. [201, 227, 228, 231, 232]

The raw fruits and vegetables can be contaminated with STH infective forms either prior to harvest are after harvest.[234] As mentioned earlier, the use of untreated wastewater and human biowaste is a significant factor for such contamination. Post-harvest contamination can occur in busy market places particularly when the market place is very busy and precludes adequate washing of the produce prior tor to distribution, or is in the vicinity of cattle markets or abattoirs.[231] For adequate decontamination of post-harvest fresh produce, methods employed include washing with chlorine, pressure washing, ultrasound application, irradiation, ozone, application of bacteriophages, antagonistic bacteria amongst others. [234] In a low resource setting, vendors are unlikely to employ any decontamination measures. At the most, they may wash the produce in water. So it is incumbent on the consumer to ensure that the fresh produce is thoroughly washed and decontaminated prior to consumption. A 2012 study in Iran by Fallah et al compared the detection rates for parasitic ova in unwashed, traditionally washed and washing by standard process of vegetables. They found that 36.6 % of the unwashed samples were positive for STH compared to 1.3% in traditionally washed and none in the standard washing process. In their study, the traditional method of washing described involved soakage of the vegetables in a sink with about 4-5 liters of water per kilogram of vegetables for the soil and mud to settles at the bottom, it was then placed in a wooden or plastic basket and then rinsed for 1-2 minutes under tap water. They used standard washing process as described by Bier involving washing of vegetables by tap water to remove mud and dust, immersion in a 200ppm solution of active calcium hypochlorite for 30 minutes for disinfection followed by rinsing in an automated fruit-vegetable washer for the final wash.[235] There is also a seasonal variation in the prevalence of contamination of fresh produce with STH infective forms with the colder months accounting for the lowest prevalence and the warmer climes for the highest prevalence, this also corresponds with the higher excretion of parasite ova during warmer climates by infected human and animal hosts favoring survival and transmission of the infective forms.[236] This has a practical importance from a public health behavioral intervention point of view. At the household level, adequate washing of raw fresh produce should constitute thorough washing with clean water and soakage in a basin with active calcium hypochlorite solution followed by rinsing. This practice needs to be followed year round but particularly in the warmer months.

2.15.3.4 Prevention of contamination of cooked food

The contamination of cooked food by infective forms of STH can occur due to multiple different mechanisms. The first being the use of garnish of inadequately washed fresh produce like carrots, lettuce, parsley, and coriander. Adequate washing as discussed earlier can prevent contamination of cooked food. The second mechanism is by use of contaminated storage containers. This can be due to contamination of the mouth and or inside of the container by the food handler or by the water used for washing the container. The water used for washing of food storage containers must be clean water and the food handler must wash hands adequately with soap and water prior to cooking food or handling cooked food.[32] Also foods with multiple ingredients are associated with higher risk of contamination due to more frequent handling.[37] A recent study by Smith et al showed that food handlers in a not for profit establishment had better food handling practices at the work place than at home even after being trained in safe food handling practices.[237] This represents an opportunity for the implementation of public health education intervention for behavioral transformation towards hand washing with soap and water for prevention and control of STH infections as envisaged by the post-2015 roadmap of WHO-UNICEF.[128]

2.15.3.5 Avoid eating food potentially contaminated with soil.

This is an intervention that is directed at the consumers. They should ensure that the food that they consume whether raw or cooked is produced under safe conditions and free of any visible contamination. This involves avoiding food sold by street vendors. Studies have shown that the knowledge and training of street vendors regarding safe food handling are not satisfactory or non-existent.[33-35] Also, eating multiple foods or foods with multiple ingredients constitutes a risk factor for infection due to increased likelihood of contamination.[37]

2.15.3.6 Washing hands contaminated with soil/feces, avoiding exposure to soil contaminated with parasite ova by using footwear, avoiding geophagy

As we have discussed above, hand washing with soap cannot be overemphasized. Gyorkos et al in their 2013 study in the Peruvian grade 5 students found that students who received public health education training and refresher sessions after deworming with albendazole had higher levels of knowledge regarding safe sanitation and had statistically significantly lower rates of re-infection with *Ascaris* infections with no difference in reinfection with *Trichuris trichiura*.[238] Also, due to the persistence of the infective forms of STH in the soil, avoiding direct contact with contaminated soil is of prime import. Hookworm larvae gain entry by penetration of intact skin and so it is essential to wear closed footwear when outside. Ngui et al in their 2012 study found that the prevalence of hookworm infection was 5.6 times higher in those who walked barefoot outdoors compared to those who used footwear for walking outside (95% CI=2.91210.73; p,0.001). [239] Geophagy is the practice of eating dirt and soil. This can be accidental or intentional. An example of accidental geophagy is when children in their toddler years and in early childhood undergo a phase of hand to mouth activity and put everything in the mouth. In the developing regions of the world, the child spends this phase outside in the courtyard in soil sharing the quarters with domestic dogs, chicken, cats, and pigs. This puts them at high risk of ingestion of the contaminated soil. Also, some mineral deficiencies can precipitate an affinity for eating dirt, which in heavily STH contaminated regions leads to heavy infections. Geissler et al and Glickman et al found geophagy to be associated higher risk of reinfection with *Ascaris lumbricoides* and *Trichuris trichiura* among children. [240, 241] It is particularly challenging to prevent a child from ingesting contaminated soil during the early childhood oral phase. Geophagy has been associated with gestation associated iron deficiency in adolescents.[242, 243] Nchito et al in their 2004 study showed a direct correlation between geophagous behavior and intensity of *Ascaris lumbricoides* infection in children in Lusaka, Zambia. In their study they did not find any difference in geophageous behavior by micronutrient or iron supplementation.[244] Adults practice intentional geophagy due to pica or as cultural practices.[245]

2.15.3.7 Eliminating other competent hosts from the human domestic environment

Mammals are the definitive hosts for STH parasites like *Ascaris*, hookworm and *Trichuris trichiura*. Generally the parasites are specific to specific animals. However, sometimes the parasites reach hosts that are not the definitive hosts. In such dead-end hosts, the parasite perishes or fails to complete the lifecycle. Sometimes, the non-definitive hosts are competent and the parasite completes its lifecycle and the cycle of transmission continues. For e.g. *Ascaris suum* which is the pig round worm can infect humans and the human round worm *Ascaris lumbricoides* can infect pigs. Both the species of Ascarids can complete their life cycles and continue transmission despite being in non-definitive hosts due to the competency of the non-definitive host.[246] Similarly, *Ancylostoma ceylanicum* and *Ancylostoma caninum* which are dog hookworms can cause infection in humans and *Ancylostoma duodenale* and *Necator americanus* which are human hookworms can infect dogs.[188] Ngui et al found that individuals who had close contact with dogs and cats had were 2.9 times more likely to have hookworm infection (95% CI=1.1927.15; p=0.009).[239] Schär et al found that the dog owners allowed their dogs to roam throughout the community and defecate indiscriminately and majority of them never picked up dog feces for safe disposal. Also, the pigs were housed in the backyard and defecated there. Some owners removed the pig feces and deposited it outside the fence. Some did not remove the pig feces daily. None of the pig owners composted the pig feces. The close proximity of these animals and their indiscriminate movements in biowaste and subsequent close contact with humans puts the humans at a very high risk for infection with STH.[190]

2.15.4 Other Supportive Activities

Other supportive activities that need to go in parallel with the strategies and interventions mentioned above are as follows:

2.15.4.1 Women's education

Women's education is a not only a social cause but also a public health intervention. Studies have shown that households of women who have attended school have better hygiene practices. Also, women when they have been taught health and hygiene behaviors are successful in implementation and adoption those behaviors by other members in the household and family.[226, 247]

2.15.4.2 Inclusion of sanitary practices in school curriculum

Incorporation of health and hygiene behaviors in the school curricula helps in the dissemination of that knowledge to other members of the household by the children Also, children are more likely to influence the adoption of such behavior by the members of the family. It helps in preventing reinfection of the children with geohelminthes after a round of MDA administered at the school.[226, 248]

2.15.4.3 Geospatial imaging systems (GIS) and remote sensing data

GIS and remote sensing are newer tools that are being increasingly used to map the prevalence of the STH infections. The mapping of the clusters also helps develop strategies for targeted delivery of MDA and WASH strategy interventions. In 2010, Global Atlas of Helminth Infections (GAHI) was inaugurated with the aim to enhance the accuracy of the distribution and determinants of diseases due to worm parasites including geohelminthes.[172] Remote sensing helps determine the geographical regions most likely to have a higher environmental prevalence of STH based on the precipitation and surface temperature fluctuation data. This helps plan strategies for environmental control of STH infections.[249]

2.15.4.4 Better surveillance by improved diagnostics

The surveillance of STH is still very resource intensive due to the necessity of taking stool samples and using older labor intensive methods for concentration, detection and identification of STH infection. This is a major limitation in the successful widespread surveillance for STH infections.[250]

2.15.4.5 Inter-sectoral collaboration

The collaboration of different sectors is of utmost necessity for successful elimination and control of STH infections. There has to be coordinated collaboration amongst sanitation engineers, toilet design engineers, water engineers, parasitologists, anthropologists, public health education professionals, school teachers and staff, drug discovery scientist, vaccine development researchers, veterinary physicians, farmers and community members. Co-ordinated collaborative actions amongst these various different sectors is essential for the strength and success of the STH control and elimination programs.[170]

2.15.4.6 Strengthening animal care services and livestock monitoring

The livestock like pigs are significant hosts for many of the human parasitic STH. Other livestock animals can also be source for zoonotic STH infections such as Oesophagostomum species that can cause human infections and have anthroponotic cycles.[208] Routine and frequent monitoring of the health of livestock is necessary. Regular treatment of livestock with preventive chemotherapy is necessary as they are at higher risk of re-infection due to habitat and dietary sources and drug resistance.

The high prevalence of hookworm disease in domestic pets such as dogs as shown in multiple studies necessitates and enhancement and strengthening of the animal care services. Programs that involve surveillance of domestic pets should be implemented in concurrence with surveillance for human disease to identify and treat domestic animal reservoirs of STH infections.[251]

Table 1: Soil borne infectious diseases (bold) and their causative agents (italics) split into two groups, "Euedaphic pathogenic organisms (EPOs)" and "Soil Transmitted Pathogens (STPs)", depending on the closeness of their relationship with soil. [56]

Euedaphic pathogenic Organisms	**Soil Transmitted Pathogens**
Actinomycetoma: (e.g. *Actinomyces israelii*)	**Poliovirus**
Anthrax: *Bacillus anthracis*	**Hantavirus**
Botulism: *Clostridium botulinium*	**Q Fever**: *Coxiella burnetii*
Campylobacteriosis: e.g. *Campylobacter jejuni*	**Ascariasis**: *Ascaris lumbricoides*
Leptospirosis: e.g. *Leptospira interrogans*	**Hookworm**: e.g. *Ancylostoma duodenale*
Listeriosis: *Listeria monocytogenes*	**Enterobiasis**(Pinworm):*Enterobius vermicularis*
Tetanus: *Clostridium tetani*	**Strongyloidiasis**: e.g. *Strongyloides Stercoralis*
Tularemia: *Francisella tularensis*	**Trichuriasis** (Whipworm): *Trichuris Trichiura*
Gas Gangrene: *Clostridium perfringens*	**Echinococcosis**: e.g. *Echinococcus multilocularis*
Yersiniosis: *Yersinia enterocolitica*	**Trichinellosis**: *Trichinella spiralis*
Aspergillosis: *Aspergillus sp.*	**Amoebiasis**: *Entamoeba histolytica*
Blastomycosis: e.g. *Blastomyces dermatitidis*	**Balantidiasis** *Balantidium coli*
Coccidioidomycosis: e.g. *Coccidiodes Immitis*	**Cryptosporidiosis**: e.g. *Cryptosporidium Parvum*
Histoplasmosis: *Histoplasma capsulatum*	**Cyclosporiasis**: *Cyclospora cayetanensis*
Sporotrichosis: *Sporothrix schenckii*	**Giardiasis**: *Giardia lamblia*
Mucormycosis: e.g. *Rhizopus sp.*	**Isosporiasis**: *Isospora belli*
Mycetoma: e.g. *Nocardia sp.*	**Toxoplasmosis**: Toxoplasma gondii
Strongyloidiasis: e.g. *Strongyloides stercoralis*	**Shigellosis**: e.g. *Shigella dysenteriae, Pseudomonas aeruginosa, Escherichia coli*
	Salmonellosis: e.g. *Salmonella enterica*

Table 2. Nutritional Disturbances during Intestinal Nematode Disturbance. [116]

Nutrient	Consequences of Parasitism	Effects on Host	Vulnerable times
Energy	Reduced Appetite, Lowered Energy intake	Inadequate energy intake to support growth, work, fetal development, school performance	Early Growth period 1-5 years, school ages, reproductive years
Protein	Slight reduction in digestion and absorption; endogenous losses may increase; reduced energy intake results in dietary protein metabolized to supply energy	Poor child growth, low infant birth weight	Early growth years, reproductive ages
Fat	Reduced absorption of dietary fat resulting in lower energy intake and inefficient absorption of Vitamin A precursors	Decreased Vitamin A availability contributes to deficiency diseases	Lactating mothers and young children
Lactose	Intestinal lactase activity reduced	Poor Lactose digestion and lactose intolerance, reduced consumption of milk	Young children
Iron	Iron loss from blood in the intestine; reduced intake from reduced appetite	Anæmia, reduced school performance, impaired work output, poor pregnancy outcome	Children and adults
Vitamin A	Reduced absorption and utilization of Vitamin A precursors	Contributes to Vitamin A deficiency	Children and adults
Other Micronutrients	Reduced food intake may result in inadequate intake of micronutrients, especially zinc, folate, and B12	Micronutrient deficiency	Children, pregnant and lactating women

Table 3. Summary of Activities for Integrated Control of Soil Transmitted Helminthes (ICSTH)

Intervention Class	**Mode or Target of intervention**	**Specific points**
Treatment of Hosts for Treatment of infection, preventive chemotherapy and Vaccination	1. Human hosts	a. Treatment of cases b. Preventive chemotherapy of specific target populations such as SAC
	2. Non-human Hosts	a. Treatment of preventive chemotherapy of domestic pets such as dogs, cats and also pigs, ruminants and non-human primates when reared close to human habitats. b. Treatment of animals in animal husbandry
	3. Vaccination	a. Humans b. Animals
Host Environment Management	1. Safe Sanitation	a. Improvements in toilet design, b. Use of additives to feces like urea, lime or ash c. Store feces for the recommended duration of time in the holding chamber prior to evacuation and use in the fields d. Centralized sewage disposal system e. Safe disposal of animal feces.
	2. Safe Water	a. Having a water treatment plant for supply of safe water b. Vigilance of water supply systems to ensure integrity and prevent contamination c. Treatment of water by heat and or filtration to ensure safety for consumption
	3. Isolation of animals	a. To minimize contact between infected animals and humans and thereby cross-infection through contaminated common environments such as soil
	4. Soil management	a. For inactivation of ova and larvae from human or non-human origin b. Treatment of soil using chemicals, fungal extracts and predatory nematophagous fungi
	5. Safe dwelling	a. Screen doors and windows to prevent access of flies and insects b. Gates to isolate livestock animals

Table 3 (Continued)

Intervention Class	Mode or Target of intervention	Specific points
Behavioral Modification for Hygiene improvement through Public Health Education	1. Hand washing	a. Use of soap every time after – i. Use of the toilet ii. Contact with animal or animal waste iii. Drinking water iv. Cooking food v. Before eating food vi. Cleaning a baby vii. Cleaning fecally contaminated surface
	2. Avoid contact with feces	a. Use of gloves when cleaning toilets, and picking up pet feces
	3. Avoid contact with contaminated soil	a. Use of closed footwear
	4. Avoid contact with contaminated water	a. Avoid wading into ponds and rivers known to have sewage discharged into them or used for open defecation

Table 3 (Continued)

Intervention Class	Mode or Target of intervention	Specific points
Behavioral Modification for Hygiene improvement through Public Health Education	5. Safe handling of animals	a. Livestock animals free-ranging around the human habitat and stabled there could have fecal contamination of skin, fur or plumage b. Particular care to be taken when milking cattle or goats for milk, wash and scrub udder or teat thoroughly Avoid children handling and playing with tails of animals
	6. Safe handling and disposal of animal wastes	a. Animal excreta should be disposed in a safe sanitary fashion. b. Dog and cat feces should be handled with protective coverings prior to cleaning c. Pet defecation areas should be isolated from children's play areas to minimize risk of contact with feces
	7. Consume safe water	a. Always consume safe water which is boiled and or filtered b. Avoid contamination of water storage containers c. Always cover water storage containers d. Use a ladle to get water from container or use a spout at the base of the container e. Do not dip fingers in water stored for drinking f. Always wash hands with soap and water before drinking water or serving water to someone else
	8. Consume Safe food	a. Always eat food cooked in hygienic and clean places b. Always keep kitchen clean and sanitized c. Cover all food to avoid contact with flies, insects and animals d. Always wash hands with soap prior to cooking, eating, serving or storing food. e. Thoroughly wash fresh vegetables and fruits to eliminate any dirt, mud and sewage residue using pressure wash and scrub them under flowing water.
	9. No Geophagy	a. Do not eat dirt b. Prevent children from eating mud or dirt
	10. No open air defecation	a. Do not practice open air defecation if access to sanitary toilet is available

Table 3 (Continued)

Intervention Class	Mode or Target of intervention	Specific points
Other Supportive Activities	1. Women's education 2. Inclusion of Sanitary practices in school curriculum 3. Geospatial Imaging Systems (GIS) and Remote Sensing Data 4. Better surveillance by improved diagnostics 5. Inter-sectoral collaboration 6. Strengthening animal care services and livestock monitoring	

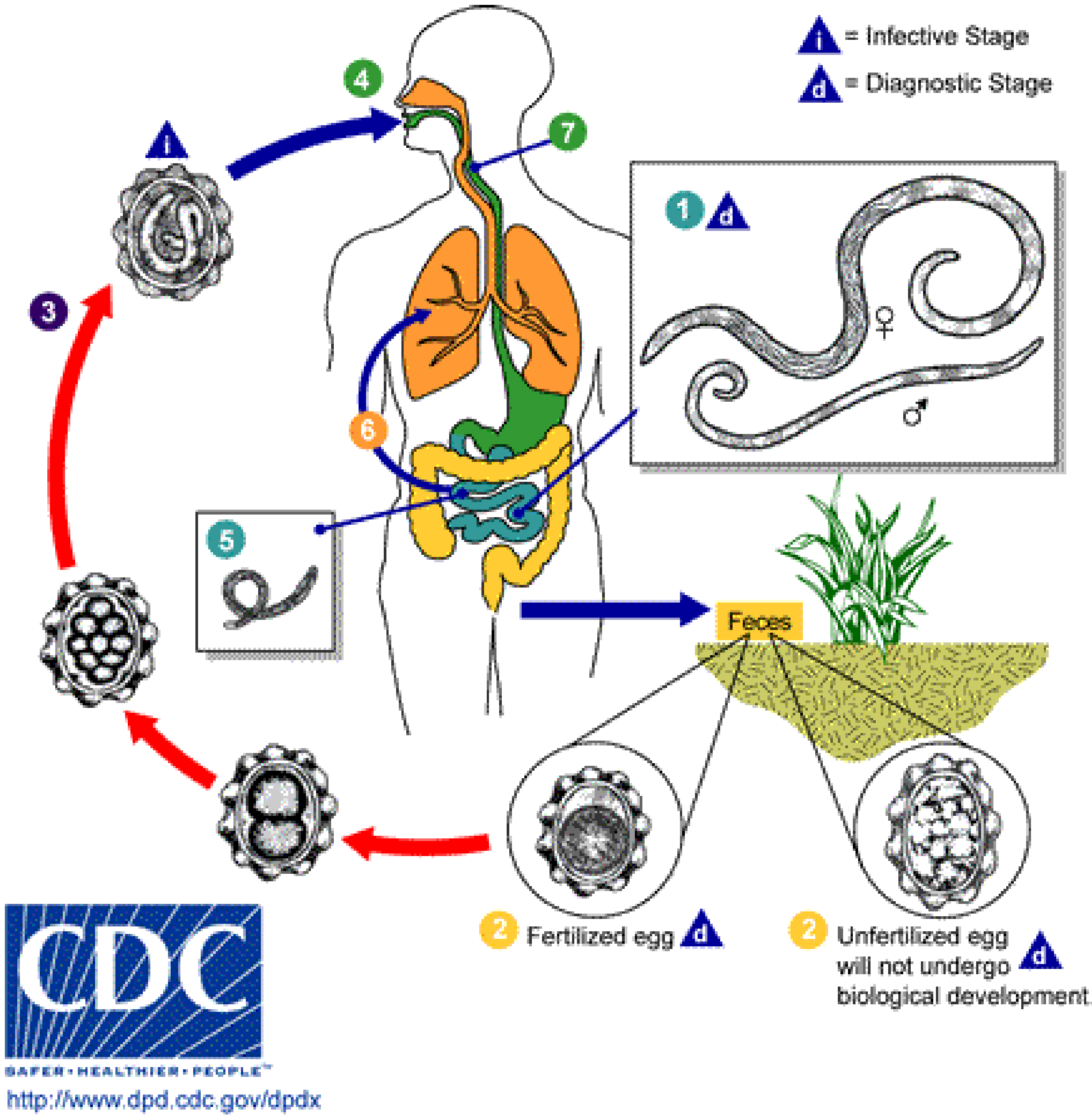

Figure 1. Life cycle of *Ascaris*[85]

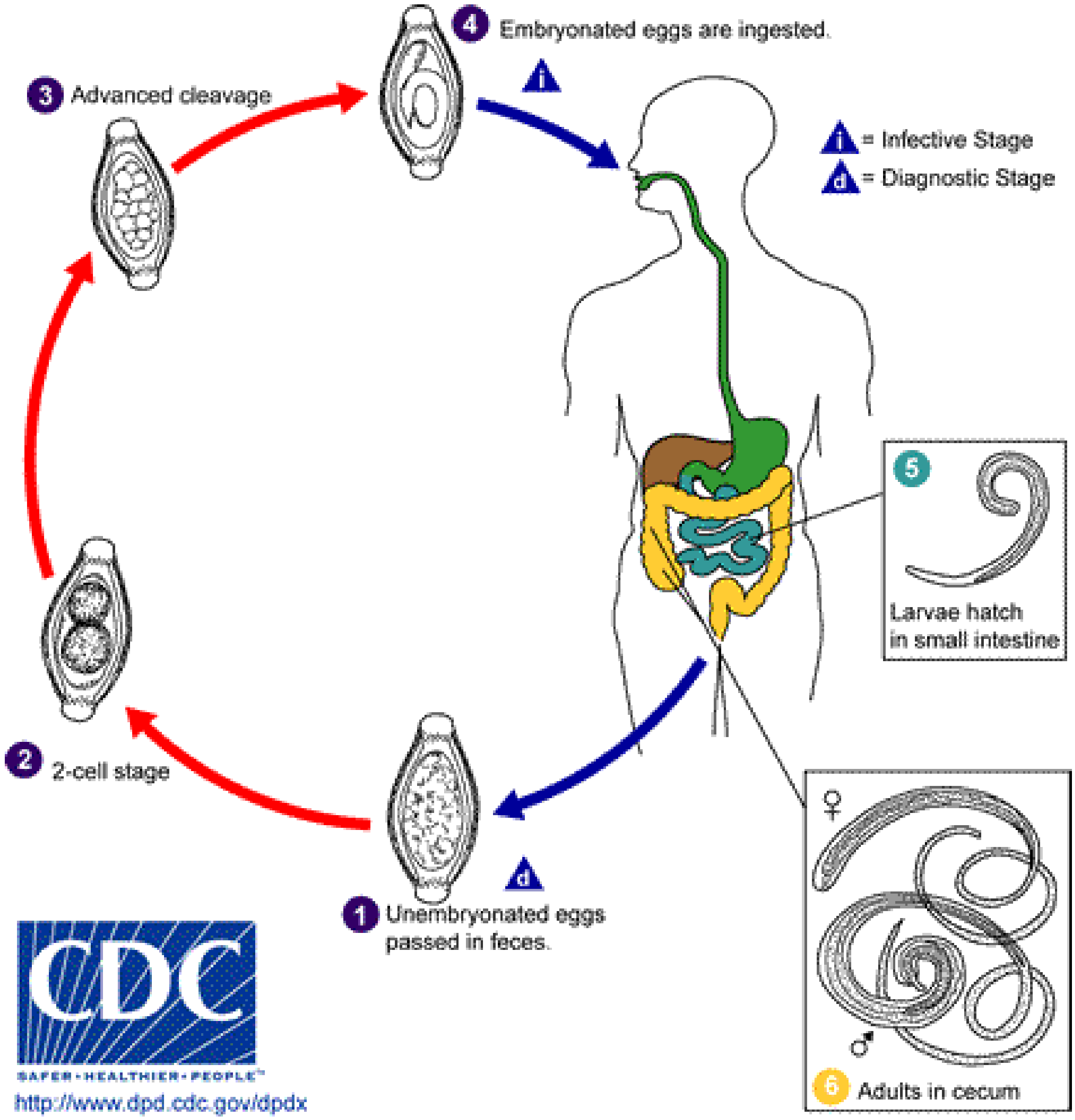

Figure 2. Life Cycle of *Trichuris*[86]

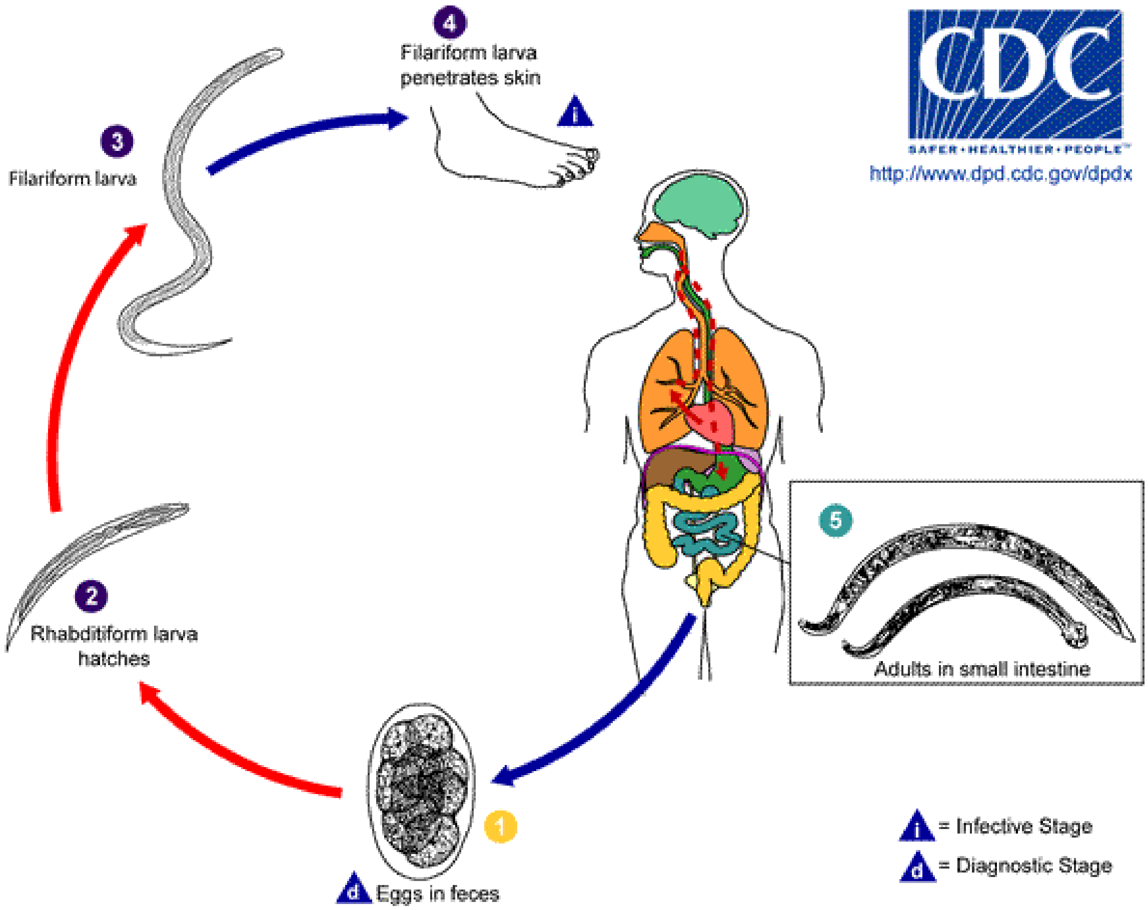

Figure 3. Life cycle of Hookworms[87]

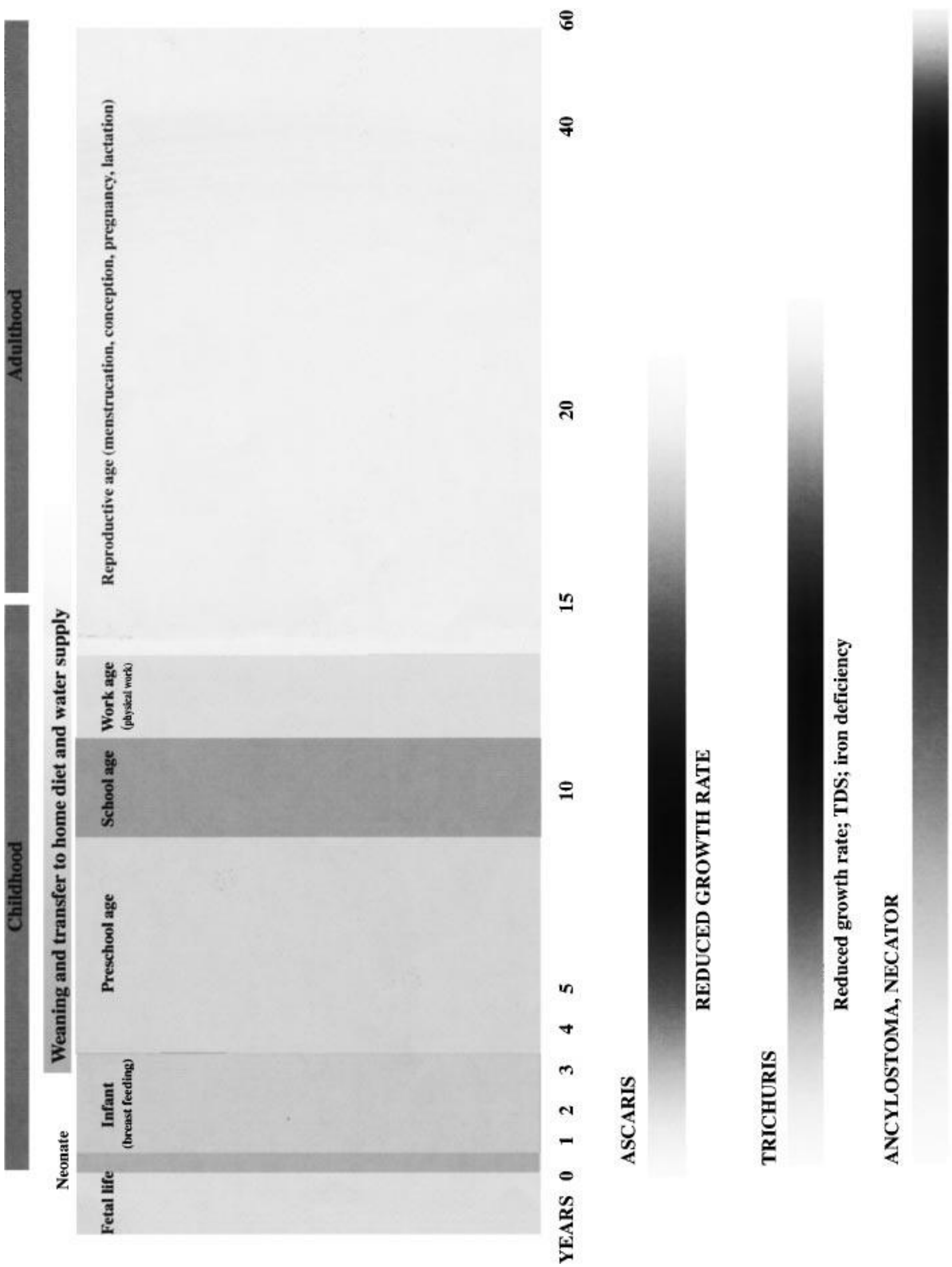

Figure 4. Soil Transmitted Helminthes and the Human Life Cycle. [116]

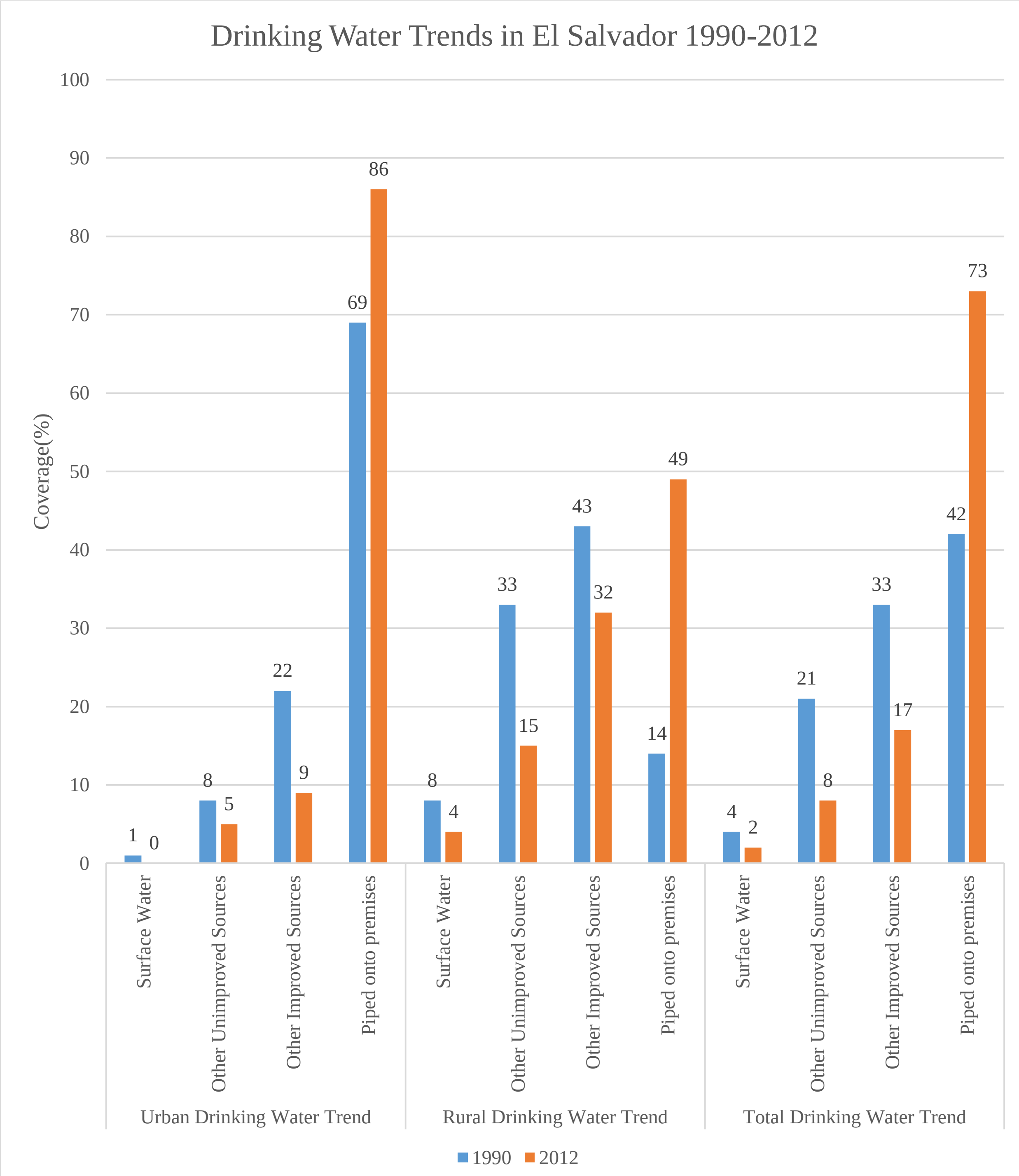

Figure 5. Urban and Rural Drinking Water trends in El Salvador-2014 estimates[1]

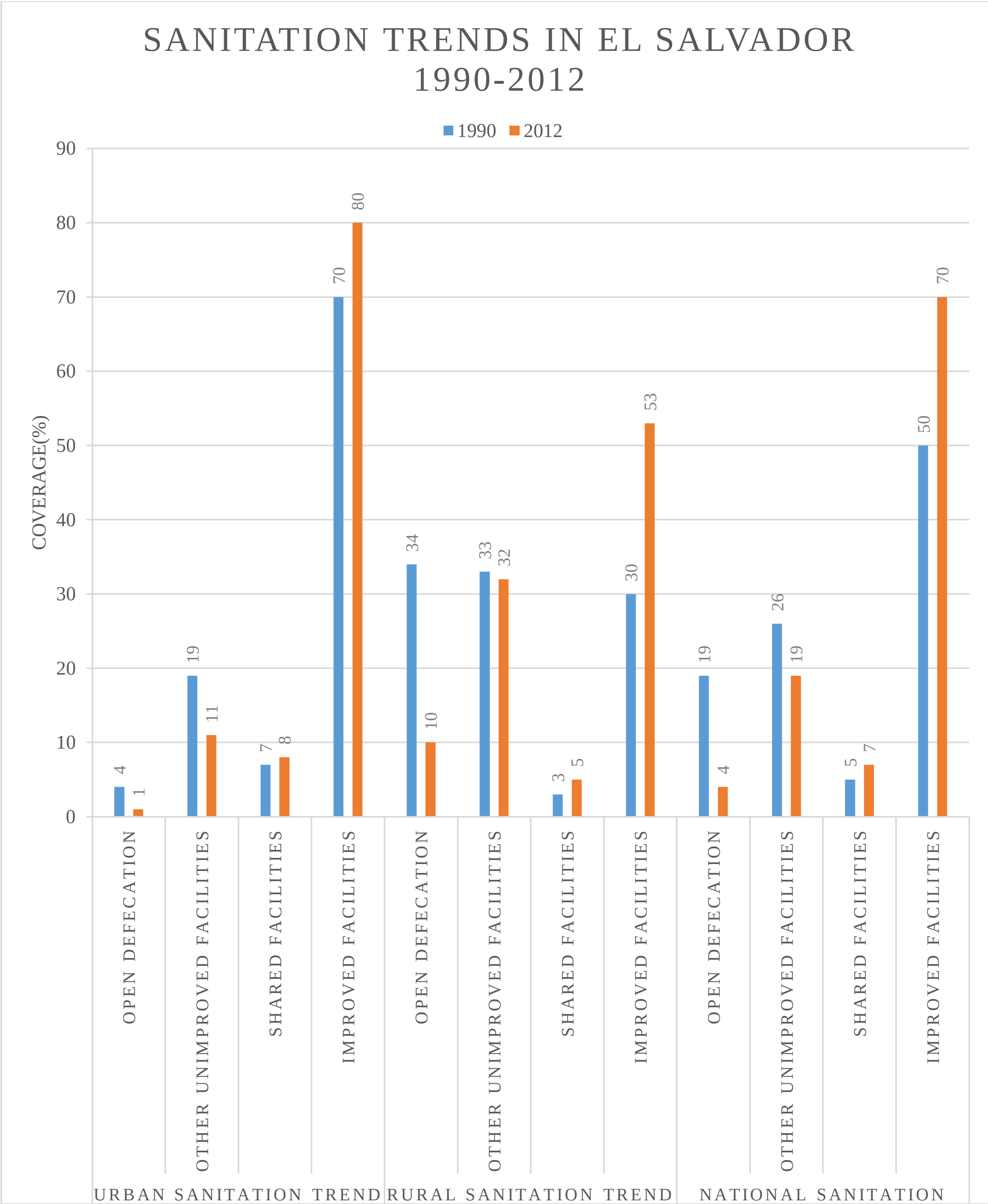

Figure 6. Urban and Rural Sanitation trends in El Salvador-2014 estimates[1]

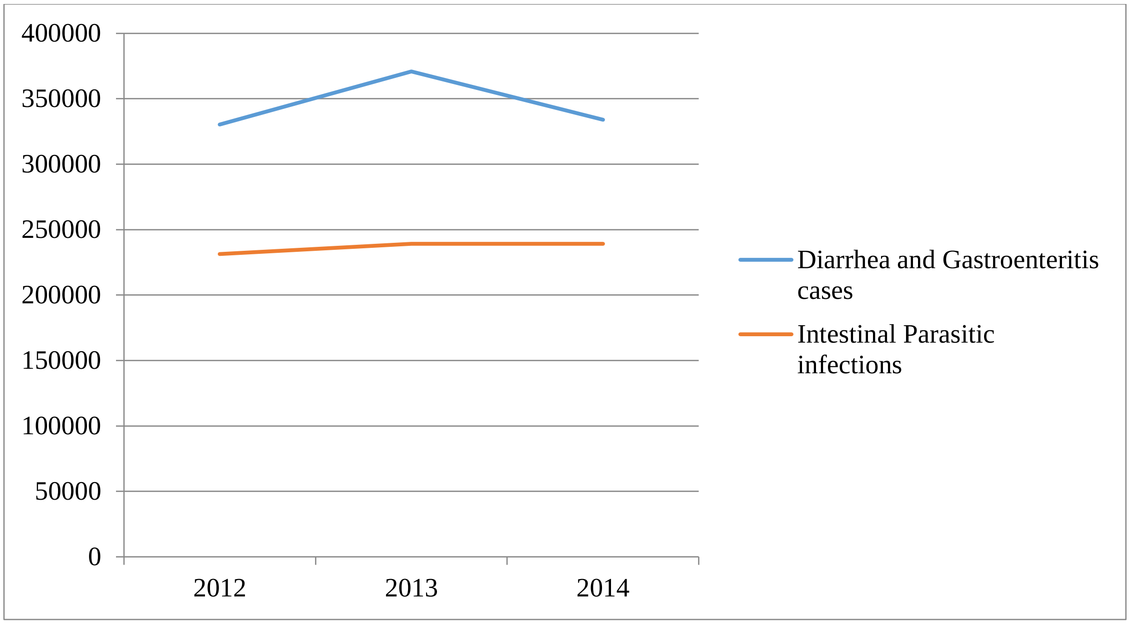

Figure 7. Current status of Diarrhea and Gastroenteritis Cases and Intestinal Parasite Infection Cases in the entire population of El Salvador.[252, 253]

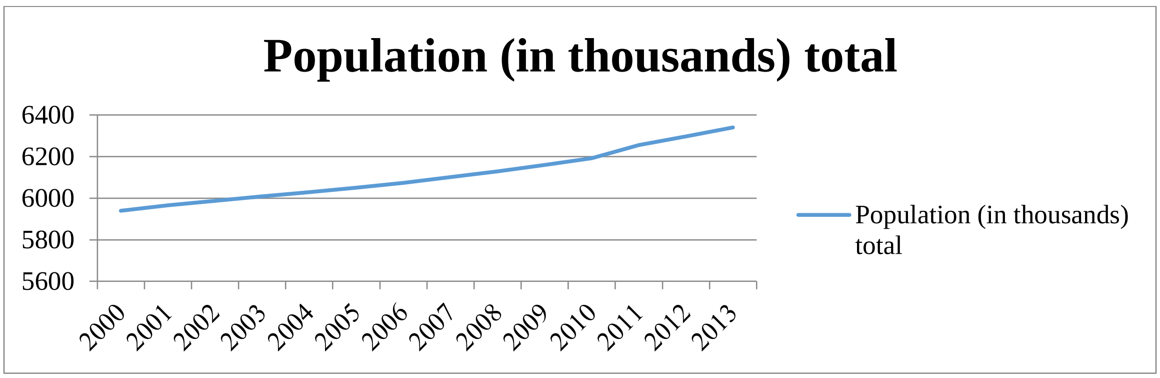

Figure 8. Population trend of El Salvador. [254]

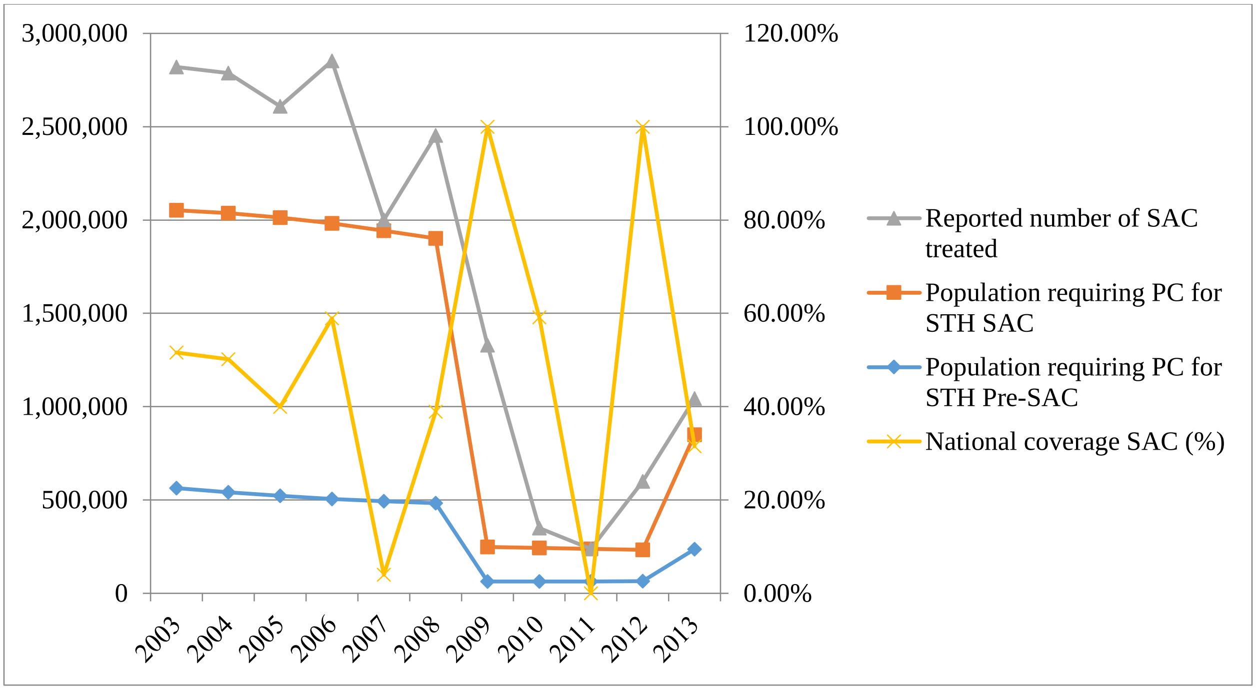

Figure 9. Trend of pediatric population requiring chemotherapy for STH in El Salvador.[255]

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CHAPTER 3:

ASSESSMENT OF PREVALENCE, RISK FACTORS AND INTERACTIONS FOR SOIL TRANSMITTED HELMINTH INFECTIONS IN SCHOOL CHILDREN IN EL SALVADOR

3.1 Abstract

3.1.1 Background

Soil Transmitted Helminth Infections (STHI) are an important group of neglected tropical diseases. The prevalence of STHI in El Salvador is not known. A national survey was carried out amongst school children to determine the prevalence and risk factors associated with their transmission in El Salvador by Ministry of Health, El Salvador (MOHES) and Pan American Health Organization (PAHO) among 8-10 year school children in 2012. We report the findings of the survey.

3.1.2 Methods

Descriptive and analytical statistics were calculated for the data of 1310 children. Individual and cumulative frequencies for infections with *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworms were calculated. Logistic regression was carried out for outcome of infection with *Ascaris*, *Trichuris*, and hookworm together. Independent variables included age, gender, eco-epidemiologic zone, urban-rural status, eating street food, poor hand washing, geophagy, use of shoes, source of drinking water and type of toilet facility. Interactions between the independent variables were tested at the multiplicative scale.

3.1.3 Results

Amongst the study participants the infection rates for *Ascaris*, *Trichuris* and hookworm were 2.75% (36/1310), 4.12% (54/1310) and 1.83% (24/1310) respectively. Significant risk for infection with *Ascaris* infection were use of shoes (p=0.0035), and eco-epidemiologic zone (p=0.0031). For *Trichuris* infection, the significant risk factors were source of drinking water (p=0.0049), use of shoes (p<.0001), urban-rural status (p=0.0219) and eco-epidemiologic zone (p<0.001). For hookworm infection the significant risk factors were use of shoes (p=0.0336) and poor handwashing (p=0.0313). There were significant interactions on the multiplicative scale amongst the independent variables for the outcomes of *Ascaris* infection, *Trichuris* infection and hookworm infection.

3.1.4 Conclusion

The low prevalence of *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm infection in the study population in the national survey of El Salvador is encouraging to attempt complete elimination of STHI in El Salvador. Significant risk factors for all type of STHI include eco-epidemiologic zones, use of shoes, source of drinking water, urban-rural status. Interactions amongst the various risk factors account for different levels of prevalence in population strata based on levels of risk factor variables. This helps us target mass drug administration and environmental control through sanitation for best optimal outcomes of infection prevention.

3.2 Introduction

STHI are an important group of neglected tropical diseases and are commonest amongst children of school going age. They mainly cause nutritional imbalances leading to poor growth and development.

El Salvador is one of the poorest countries in Central America with previous studies reporting significant burden of STHI.[1] The limited resources make addressal of neglected tropical diseases challenging. Despite that, El Salvador has made significant progress towards the achievement of the

Millennium Development Goals for water and Sanitation. The 2014 Joint Monitoring Program of the WHO and UNICEF for Water Supply and Sanitation (JMP-WHO/UNICEF for WSS) estimates for El Salvador show that 10% of the country still relies on unimproved water sources for their water needs. The urban-rural difference in the coverage of piped water is significant in El Salvador. In the urban regions there is 86% coverage while in the rural parts, the coverage with piped water is only 49%. Also, while 5% of the dependence in the urban regions is on unimproved sources, in the rural regions it is 19%.[2, 3] Regarding access to safe sanitation, the coverage of improved sanitation facilities is only 70% nationwide and still nearly a quarter of the country still uses unimproved sanitation facilities with about 4% practicing open defecation. The urban-rural difference in the coverage with improved sanitation is 80% for urban versus 53% for rural areas. Also, while 9% of the urban region still uses unimproved sanitation facilities, in the rural regions it is 42%.[2]

Soil transmitted helminths (STH) infect humans in all age groups. However, the highest prevalence is in the school aged children. Previous study by Corrales identified a 12 fold risk for children aged 4-12 years of age for infection with STH compared to other age groups. Their study reported prevalence rates of 8% for *Ascaris*, 20.9% for *Trichuris* and 22.3% for hookworms.[1] Also, targeted therapy at this age group is more successful in reducing prevalence of STHI.[4] Amongst the reason that children have a higher burden of disease with STHI include behavioral factors, developmental factors and educational factors. Studies have shown that children during their developmental years as a toddler tend to put things in the mouth later in school age play with soil.[5] This is fraught with risk of ingestion of infective forms of STH like ova of *Ascaris lumbricoides* and *Trichuris trichiura* particularly in regions where the peridomestic environment of the habitation is also shared with domestic animals like dogs, pigs and other animals. Mammals are the definitive hosts for STH parasites like *Ascaris*, hookworm and *Trichuris trichiura*. Generally the parasites are host-specific. However, sometimes the parasites reach hosts that are not the definitive hosts. In such dead-end hosts, the parasite perishes or fails to complete the lifecycle. Sometimes, the non-definitive hosts are competent and the parasite completes its lifecycle and the cycle of transmission continues. For e.g. *Ascaris suum* which is the pig round worm can infect humans and the human round worm *Ascaris lumbricoides* can infect pigs. Both the species of *Ascaris* can complete their life cycles and continue transmission despite being in non-definitive hosts due to the competency of the non-definitive host.[6] Similarly, *Ancylostoma ceylanicum* and *Ancylostoma caninum* which are dog hookworms can cause infection in humans and *Ancylostoma duodenale* and *Necator americanus* which are human hookworms can infect dogs.[7] Ngui *et al* found that individuals who had close contact with dogs and cats had were 2.9 times more likely to have hookworm infection (95% CI=1.1927.15; p=0.009).[8] Schär *et al* found that the dog owners allowed their dogs to roam throughout the community and defecate indiscriminately and majority of them never picked up dog feces for safe disposal. Also, the pigs were housed in the backyard and defecated there. Some owners removed the pig feces and deposited it outside the fence. Some did not remove the pig feces daily. None of the pig owners composted the pig feces. The close proximity of these animals and their indiscriminate movements in biowaste and subsequent close contact with humans puts the children at a very high risk for infection with STH.[9] Also, lack of safe toilet facilities usually results in the children evacuating in the yard around the house indiscriminately. This leads to contamination of the yard soil with ova and larvae of STHI and subsequently leads to infection of the children as they play and spend time in the yard.

Behavioral factors responsible for transmission of STHI include poor hand washing, eating street food, geophagy and use of footwear. Proper handwashing reduces the risk of STHI along with use of footwear. Use of footwear helps avoid direct contact with contaminated soil. Hookworm larvae gain entry by penetration of intact skin and higher hookworm infections rates are found in those who do not use footwear.[8] Gyorkos *et al* in their 2013 study in the Peruvian grade 5 students found that students who were received public health education training and refresher sessions after deworming with albendazole had higher levels of knowledge regarding safe sanitation and had statistically significantly lower rates of re-infection with *Ascaris* infections with no difference in reinfection with *Trichuris trichiura*.[10] Also, due to the persistence of the infective forms of STH in the soil, avoiding direct contact with contaminated soil is of prime importance. Hookworm larvae gain entry by penetration of intact skin and so it is essential to wear closed footwear when outside. Ngui *et al* in their 2012 study found that the prevalence of hookworm infection was 5.6 times higher in those who walked barefoot outdoors compared to those who used footwear for walking outside (95% CI=2.91210.73; p,0.001). [8]

Geophagy is the practice of eating dirt and soil. This can be accidental or intentional. An example of accidental geophagy is when children in their toddler years and in early childhood undergo a phase of hand to mouth activity and put everything in the mouth. In the developing regions of the world, the child spends this phase outside in courtyard sharing the quarters with domestic dogs, chicken, cats, and pigs and their mutual soilings. This puts them at high risk of ingestion of the contaminated soil. In heavily STH contaminated regions, geophagy leads to heavy infections. Geissler *et al* and Glickman *et al* found geophagy to be associated higher risk of reinfection with *Ascaris lumbricoides* and *Trichuris trichiura* among children. [11, 12] It is particularly challenging to prevent a child from ingesting contaminated soil during the early childhood oral phase. Geophagy has been associated with gestation associated iron deficiency in adolescents.[13, 14] Nchito in their 2004 study showed a direct correlation between geophagous behavior and intensity of *Ascaris lumbricoides* infection in children in Lusaka, Zambia. [15]

Street food is generally unsafe due to inadequate preparation or contamination due to unhygienic practices of food handlers and poses a risk for STHI. Studies have shown that the knowledge and training of street vendors regarding safe food handling are not satisfactory or non-existent.[16-18] Also, eating multiple foods or foods with multiple ingredients constitutes a risk factor for infection due to increased likelihood of contamination.[19]

Another possible explanation for higher infection rates in children could be the immune response elicited by STH. There is Th2 type of immune response due to infection with STH which is characterized by increased secretion of type 2 immune cytokines like IL-4 and IL13. Also, memory T-cell are formed that lead to increased secretion of these Th2 immune response markers upon re-infection. However, the immune offers very little protection against re-infection primarily against *Trichuris trichiura*. A study looking at re-infection with hookworms found increased levels of IL-5.[20, 21]

Ecology plays a significant role in the maintenance and transmission of STH. The survival of the STH in the environment is dependent on the ambient temperature, pH and water content of the soil. They thrive in tropical and subtropical regions of the world as they need the warm moist climate for their development in the soil.[22] The egg forms of the some STH can survive desiccation such as those of *Ascaris*, however, the eggs of hookworms are fragile and it is the larvae that are the infective forms. The prevalence of STH infections is found to be lower in the mountains than in the plains at sea level due to the altitudinal effect on ambient temperature. Also, the rains in the mountains tend to wash away the eggs with the soil to the lower regions.[23] *Ascaris* eggs persist, survive and mature faster becoming embryonated when in clay soil compared to sandy soil under shade. Sandy soil does the same for hookworm larvae.[24]

3.3 Methods

In 2012 the study 'Prevalence and intensity of geohelminth infections and carriers of malaria prevalence in school children aged 8-10 years in El Salvador, 2011' was carried out. This study was a collaboration of the Ministry of Health of El Salvador (MOHES), Ministry of Education of El Salvador (MOE) and World Health Organization (WHO)/Pan American Health Organization (PAHO). The study was funded by the National Budget of the Ministry of Health of El Salvador, National Budget of the Ministry of Education of El Salvador and the PAHO Disease Prevention and Control Project.[25]

The general objective of the original study was to determine the prevalence and intensity of infection with helminths Transmitted by contact with soil (STH) in school age children in El Salvador. The specific objectives were:

a. To determine the national cumulative prevalence and intensity of infection for STH i.e. *Ascaris lumbricoides*, *Trichuris trichiura* and hookworms in school aged children.
b. To determine the prevalence of infection with STH in Eco-epidemiologic zones for *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm in school aged children.

c. To determine the prevalence of STH infection by parasitic individual species i.e. *Ascaris lumbricoides*, *Trichuris trichiura* and hookworms in school aged children.

3.3.1 Sample Design

This was a multistage prevalence study, with proportional allocation of clusters and by the presence of municipalities, Community Equipment Health (ECOS) for each of the five targeted eco-epidemiological zones, with both random selections of schools within clusters and of individuals within those municipalities was done taking into account the environmental elements, socioeconomic and demographic variables that possibly influence the prevalence of STH.

3.3.2 Inclusion and Exclusion Criteria

3.3.2.1 Inclusion criteria

a. Be third grader primary education enrolled in public or private school, urban rural country or attached to the MOE.

b. No discrimination of sex, race, political or religious beliefs

c. Personally come to deliver the stool sample

d. Provide informed consent signed by parents

e. Did not receive any antiparasitic treatment in the days prior to sampling

f. Stool sample of hard or pasty consistency delivered on the sample collection day

g. Answered the sample collection form and evaluation of associated factors

3.3.2.2 Exclusion Criteria

a. The student did not personally come to school on the day specified for delivery / collection of sample.

b. The child did not have the signed informed consent from parents.

c. Stool sample was soft or liquid consistency as it made it impossible to process the sample by the Kato-Katz method

d. Did not answer the questions on the form for sample collection and evaluation of associated factors for STH infection risk.

A total of 1325 children participated in the study. [25]

3.3.3 Data Management and Statistical Analyses

After collection in the field, data was cleaned and managed using the SAS v9.4 (Cary, NC) statistical analysis software. Nineteen pairs of records were found to have the same code number. These were then compared in sequential order of variables and 18 records were assigned a new code number due to distinctness. Two record were deleted. Some observations with values of 'Other' and 'Unknown' for the variable 'source of water' did not have any parasite positivity and were deleted to lower the number of categories and increase the power of bivariate analyses. The percentage of deleted observations was 13/1323 which was less than 1% of the total number of samples and so did not have any effect on the further analyses of the data. The final analyses were carried out on the records from 1310 subjects. A new variable 'rural-urban status' was created based on the data from the census of El Salvador. There is no definition of when an area is determined to be urban or rural although San Salvador is known to be urban. Protocol of the MOHES and PAHO for the data collection for the survey mentions including San Salvador as an independent site at par with the other departments to have a comparison with an urban location and because of its population density. From the 2007 Population Census of El Salvador, the ratio of urban to total population for each municipality was determined.[26] If it was greater than 50% then the cantonment was determined to be urban, otherwise it was designated as rural. This was done to determine if there was any association between the rural-urban location of the study subjects and their infection with soil transmitted helminthes. Some of the categorical independent variables were condensed into fewer categories to retain power for statistical analyses. They include 'use of shoes' and 'type of toilet facility'. 'Use of shoes' was condensed from originally five categories to three categories. Specifically, the categories of 'nothing, 'always sandals' and 'sometimes sandals' were merged to create 'open footwear or nothing'. The other categories 'always closed shoes' and 'sometimes closed shoes' were retained as such. 'Type of toilet facility' was condensed from originally five categories to three categories. The categories of 'septic tank' and 'flush toilet' were merged to create 'septic tank or flush toilet' as they are an improved type of sanitation facility. The categories of 'composting latrine' and 'latrine' were merged to create 'some latrine' which is a lower form of sanitation facility than the previous merged category. 'Open defecation' was retained as such and is the lowest type of sanitation facility. The original dataset had binary variables for sources of drinking water like 'piped water', 'well' and 'packaged water'. Also, there was a variable that listed 'other sources of drinking water'. We consolidated all these different variables pertaining to source of drinking water into 4 categories by creating a new variable 'source of drinking water'. The four categories were 'piped water +/- non-chlorinated water', 'spring or well', 'packaged water' and 'not-chlorinated water'. The assumption here is that individuals with access to piped water would prefer to use piped water rather than not-chlorinated water for drinking purposes. 'Spring' and 'well' are merged as they are both surface water sources.

Descriptive statistics were generated using the PROC FREQ procedure and Chi-square tests done to identify significant associations amongst the variables. Outcome variables were identified as the occurrence of infection with *Ascaris*, *Trichuris* and hookworm infection based on the identification of ova and or cysts in the stool samples. The Logistic regression was carried out in two stages using the PROC LOGISTIC procedure. The first was unadjusted logistic regression, followed by fully adjusted logistic regression. Collinearity testing was carried out for the independent variable using the method described by Allison.[27] Based on bio-behavioral plausibility seven possible two-way interactions were identified (Table 4). The interactions were tested on the multiplicative scale as well as the additive scale. For testing on the multiplicative scale, a single interaction pair was added to the fully adjusted model and the odds ratio estimates of the interaction term were determined. For interaction testing on the additive scale, the categorical independent variables were tested taking two levels of each of the variable at a time and Relative Excessive Risk due to Interaction (RERI) was calculated with 95% CI. [28] Significant multiplicative interactions were determined by calculating the odds ratios and 95% confidence intervals of the interaction term in logistic regression in fully adjusted models. Significant additive interactions were determined by calculating the Relative Excess Risk due to Interaction (RERI) and 95% confidence interval for each of the seven pairs of plausible interactions take two levels of each main effect at a time.

3.4 Results

Of the 1310 children 49.01% (n=642) were male and 50.99% (n=668) were female. The age distribution of the children that participated in the study was 30.46% (n=399) 8 year olds, 43.89% (n=575) 9 year olds and 25.65% (n=336) 10 year olds. Most of the children were from the central depression region (286, 21.83%) of El Salvador. This is because the capital San Salvador which was added as the sixth unit lies in the central depression region. They were followed by 277 (21.15%) children in the coastal plains, 250 (19.08%) each in the mountain and volcanic chain regions and 247 (18.85%) in the coastal chain region. Residents of rural areas comprised 81.98% (n=1074) of the study population versus 18.02% (n=236) in the urban areas. The sources of drinking water and toilet facility were as shown in Table 5 and Table 6. Poor hand washing was reported by 495 (37.79%) of the subjects, eating street food was reported by 890 (67.94%) and geophagy was reported by 28 (2.14%) of the subjects. Three hundred and sixty three (27.71%) subjects reported using footwear always while 606 (46.26%) wore sometimes closed shoes and 341 (26.03%) wore sandals or nothing at all.

Ascaris infection was identified in 36 (2.75%) subjects, *Trichuris* infection in 54 (4.12%) and hookworm infection was identified in 24 (1.83%). Significant associations were found by chi-square test for the outcome variables. The p-values form chi-square tests for each of the four outcomes is shown in Table 7. Tables 8 gives the significant odds ratios with the 95% confidence interval from logistic regression analyses for the independent variables for *Ascaris* infection in unadjusted and fully adjusted models, while Table 9 does the same for *Trichuris* and Table 10 for hookworm infection. Collinearity testing did not identify any significant collinearity amongst any of the independent variables. The test results of interactions testing on the multiplicative scale is given in Tables 11 for *Ascaris* infection, Table 12 for hookworm infection and Table 13 for *Trichuris* infection. The RERI scores for the interaction tests on the additive scale are given in Tables 14 for *Ascaris* infection, Table 15 for Hookworm infection and Table 16 for *Trichuris* infection.

3.5 Discussion

STHI are an important group of neglected tropical diseases. *Ascaris lumbricoides*, *Trichuris trichiura* and hookworms- *Necator americanus* and *Ancylostoma duodenale* form the major bulk of infections worldwide.

3.5.1 Age and Gender

The prevalence rate for *Ascaris* infection in our study sample is 2.75% while for *Trichuris trichiura* it is 4.12% and for hookworm infection it is 1.83%. Sorto et al in their 2015 analyses of the same database as us reported an infection rate of 2.7% for *Ascaris*, 4.1% for *Trichuris trichiura* and 1.8% for hookworm.[29] The minor difference in the results is possibly due to our different data management strategy. This prevalence rate is lower than the previously published reports on STH studies in El Salvador. In 1988, Reinthaler *et al* in their study looking at the stool samples of 210 children with diarrhea identified intestinal helminthes and protozoa in 49% of the children. Amongst the helminthes infections, 31% were *Trichuris trichiura*, 18% were *Ascaris lumbricoides* and 5 % were *Hymenolepis nana*.[30] In 2006, Corrales *et al* found that 53% of their study population had intestinal parasitic infections.[1] Of those *Ascaris lumbricoides* accounted for 8%, *Trichuris trichiura* for 22.9% and hookworms for 20.3%. Children in the age-group 6-12 years had 1.5 times higher risk for infection with *Ascaris* when compared to other age groups and they also had the highest percentage of infection with *Trichuris trichiura* when compared to all age groups. Our study looks at children in the 8-10 years age group. Also, in 2006, Corrales *et al* found that males had a higher prevalence for infections with *Ascaris*, *Trichuris* or hookworms when compared to females and agricultural occupations were associated with higher hookworm infections. In our study there was no statistically significant gender-wise difference in the prevalence of infections by *Ascaris*, *Trichuris* and hookworms. However, in contrast to the findings by Corrales *et al*, in our study girls had higher infection rate (1.45%) compared to boys (1.30%) for *Ascaris* and also for hookworm (1.15% girls vs 0.69% boys). For *Trichuris* infections boys had a higher number compared to girls in our study. This is similar to their findings.

3.5.2 Rural-urban Status

The urbanization status of a region has been shown to be associated with infection rates of STH. In our study we found that the rural status had a protective effect on infections with *Trichuris* infection [OR 0.414 (0.196-0.877)] and hookworm infection [OR 0.307 (0.095-0.989)]. The association was not significant for infection with *Ascaris*. The reason for a protective effect of rural regions could be due to lesser population density compared to the cities. This is in contrast to the findings of Pullan and Brooker in their 2012 global study where they found that urban regions were associated with lower prevalence of hookworm infections compared to rural areas. For *Ascaris* and *Trichuris* infections they found a higher risk with urban areas. This is different from our findings for *Ascaris* but similar to our findings for *Trichuris* infections. Fenghua in their 1988 study in Jiangsu province of China found that the number of infections due to STH decreased with economic development with higher rates observed in the less economically developed and more agrarian Pizhou county compared to Wujiang county.[31] Phiri *et al* found that STH infections were more prevalent in urban areas.[32] Mahmud in their 2013 study on children in northern Ethiopia reported higher prevalence of *Ascaris* and *Trichuris* infection in children in the urban areas while hookworm infection was highest in the rural areas. [33]

3.5.3 Eco-epidemiologic Zones

We found that there was a significant association between the ecological zone and STHI. For *Ascaris* infection, the significant eco-epidemiological odds ratios were for the central depression region [OR 10.500 (2.244 – 49.123)] compared to mountain. For *Trichuris* the only significant odds ratio was for the coastal plains [OR 31.946 (4.142 – 246.415)] while for hookworm there was none. Sorto et al found similar findings except, they reported that the region with highest prevalence of hookworm infections was volcanic chain at 2.4% and coastal chain at 2.1%. We found similar results but there was no statistically significant difference in the odds ratios for different eco-epidemiologic zones with respect to infection with hookworm. This difference in the odds ratios for the different eco-epidemiologic zones could be possibly due to the differences in the climatic temperature, soil type and soil moisture levels. This is in agreement to the findings of 2011 study by Gunawardena et al who found altitudes above 500m to have a protective effect from infections with *Ascaris lumbricoides*, *Trichuris trichiura* and hookworms.[34] The mountainous regions due to the altitudinal effect have less ambient conditions of temperature and the rainfall there washes the soil with eggs to low lying areas. The higher odds ratios can be explained by the study done the 1952 study done by Beaver that reported clayey soil favors the development and maturation of *Ascaris* while sandy soil does the same for hookworm larvae.[24] In a 2003 study in South Africa, Mabaso *et al* found that the prevalence of hookworm transmission was highest in regions ≤ 150 m in the sandy coastal plains compared to the mountains.[23] Thus, there is a need to study STHI prevalence with type of soil considering these parasites need to complete their development in soil.

3.5.4 Source of Drinking Water

Water is an important factor when studying STH. We found that having a source of drinking water as ‘spring or well’ compared to piped water was associated with much high odds ratios for infection with *Ascaris* [OR 3.199 (1.377 – 7.430)] and *Trichuris* [3.105 (1.541 – 6.253)]. Spring and well water are surface water sources. These are not secure and have higher risk of contamination from surface run-off due to rainfall and human activity and sewage.[35] This compromises their quality posing a higher risk for transmission of food and water-borne pathogens. These water sources also could serve as irrigation sources and their contaminated waters then can contaminate the fresh produce, which if consumed raw increases risk of infection. There was no statistically significant odds ratio for hookworm infection that was associated with source of drinking water. Sorto et al reported that the likelihood of infection with *Ascaris lumbricoides* was high [OR 3.9 (1.7 – 9.3)] when the child practiced open defecation, consumed well water and did not use piped water as a source of drinking water. For infection with *Trichuris trichiura*, they report that the infection was higher in children who did not use ‘piped water’ as source of drinking water [OR 2.3 (1.3-3.9)] and used well as source of drinking water [OR 3.2 (1.1-9.1)]. They did not report any association between source of drinking water and hookworm infection. For risk of STHI on the whole, they found that the children who used well as source of drinking water were 2.7 times more likely than those who used other sources of water for drinking. They also found that drinking piped water was protective against STHI infection when compared to all other sources of water for drinking purposes. [29]

3.5.5 Type of Toilet Facility

Our study found significant odds ratio associated with intestinal parasite infection [OR 2.408 (1.508 – 3.846)] for those practicing open air defecation compared to those using a septic tank or flush toilet. For *Ascaris* infection, the odds ratio was significant in the unadjusted model [4.216 (1.706 – 10.41)] for those practicing open air defecation compared to septic tank or flush toilet but lost significance in the fully adjusted model. There was no significant odds ratio for infection with *Trichuris* and hookworm. Similarly, Sorto et al also found that children who practiced open air defecation had higher risk of STHI [OR 2.2 (1.1-4.2)]. Open air defecation is a significant risk factor for infection with STH due to potential for contamination surface water, body surface and exposure to flies that can mechanically transfer ova of *Ascaris*, *Trichuris* and hookworms.[36] Sorto et al reported that children practicing open air defecation were three times more likely to have STHI than those who did not. Children practicing open air defecation near the bushes in Ethiopia were reported to have higher infection rates for hookworm.[33] For *Trichuris* infection our findings are in accordance to those of Gabrie *et al* in their 2014 study in Honduras where they did not find any association between infection with *Ascaris* and *Trichuris* and absence of sanitation facilities at home, but contrast for *Ascaris* infection.[37] Corrales *et al* in their 2006 study evaluating the impact of sanitation type on STH found that the odds ratios for infection with *Ascaris*, *Trichuris* and hookworm for the Double Vault Urine Diversion (DVUD) toilet compared to the solar toilet and only for *Ascaris* and *Trichuris* infection when compared to the pit latrine. Solar latrines were found to be protective against infection with *Ascaris*, *Trichuris* and hookworms.[1] Based on the studies carried by Corrales *et al*, the MOHES in 2005 emitted a sanitary regulation enforcing populations to bury all DVUD contents. This plus the extensive school based deworming program may have had an important impact in reducing the prevalence of STHI in El Salvador.[1, 38]

3.5.6 Poor Handwashing

We found that while poor hand washing had no significant odds ratios for infection with *Ascaris* and *Trichuris*, it was protective for infection with hookworm [OR 0.175 (0.043 – 0.714)]. Poor hand hygiene has been linked with higher hookworm infection in children.[33] Gyorkos *et al* in their 2013 study in the Peruvian grade 5 students found that students who were received public health education training and refresher sessions after deworming with albendazole had higher levels of knowledge regarding safe sanitation and had statistically significantly lower rates of re-infection with *Ascaris* infections with no difference in reinfection with *Trichuris trichiura*.[10] As mentioned, El Salvador has regular school based deworming campaigns that are targeted primarily at school age children. Our results could possibly be explained by the success of the deworming campaign regardless the lack of improvement in handwashing. Fung in their review of literature did not find any conclusive effect of protection of handwashing against *Ascaris* infection. They did however find a protective effect of use of soap for handwashing to be protective against the prevalence of *Ascaris* infection but not on intensity.[39]

3.5.6 Eating Street Food

We did not find any statistically significant association between eating street food behavior and infection with *Ascaris*, *Trichuris* and hookworms. This is in contrast to the findings of Tadesse in their 2005 study in Ethiopia that found a higher prevalence of infection with *Ascaris* and *Trichuris* in children eating street food.[40] This difference could be possible due to better food handling practices by street food vendors in El Salvador.

3.5.7 Geophagy

We did not find any statistically significant association between geophagy and geohelminth infection. However, it did emerge as a protective for infection with intestinal parasites [OR 0.128 (0.024 – 0.670)]. This intriguing finding is similar to the one reported by Freeman et al in their 2013 cluster randomized trial assessing the impact of school based water hygiene, water quality and sanitation intervention on STH re-infection, where they identified geophagy to be protective against infection with *Trichuris trichiura* [RR 0.33 (0.12-0.92)] compared to controls.[41] Our findings are in contrast to those of Geissler *et al* who determined a causal association between geophagy and infection with *Ascaris* and *Trichuris*.[11] However, in agreement to other reports of geophagy not being a risk factor for geohelminth infection and that the risk potential of geophagy depends soil contamination by feces and the parasite content of the consumed earth.[42, 43]

3.5.8 Use of Shoes

Numerous studies have shown that behavior pattern of use of shoes is associated with geohelminth infection. We found in our analyses that use of sandals or nothing versus always wearing closed shoes was associated with a significantly higher risk of infection for *Ascaris* [OR 2.582 (1.051 – 6.342)] and *Trichuris* [OR 4.297 (1.827 – 10.105)]. For hookworm the odds ratio was significant in the unadjusted model [OR 8.697 (1.082 – 69.90)] but not in the fully adjusted logistic regression model. The use of closed shoes some of the times was also protective against hookworm infection [OR 8.647 (1.117 – 66.962)]. As the infective larvae of hookworm penetrate through bare skin, the association of closed footwear all the time or some of the time is significantly protective against hookworm infection compared to wearing of open footwear or nothing. Jiraanankul in their 2011 study similarly found high risk of hookworm infection with barefoot walking.[44] Sorto et al in their analyses reported that children who did not wear footwear all the time had a higher risk of STHI [OR 2.1 (1.2-3.7)].[29]

There are very few studies that test for interactions of risk factors for STHI. Below are the results of interaction assessment for the three STH studied and the possible explanations:

3.5.9 Testing For Interactions on Multiplicative Scale and Additive Scale For *Ascaris* Infection

3.5.9.1 Interactions for Ascaris infection on multiplicative scale (Table 11)

The use of open footwear or bare-feet walking was associated with nearly three times the risk of wearing closed footwear when an individual did not practice geophagy. Conversely in urban locales, use of closed shoes some of the times compared to open footwear or nothing was found to be protective against *Ascaris* infection as compared to rural areas. This could be due to the higher population density in the urban areas and poor sewage and waste disposal thereby increasing risk of infection with open footwear or bare feet walking. Water source was found to play a significant role in the prevalence of *Ascaris*. Poor hand washing was very highly associated with *Ascaris* infection particularly when the source of drinking water was 'spring or well' water. This could be possible due to the contamination of these surface water sources with sewage. To further support the idea, the risk of *Ascaris* infection was greatly elevated with consumption of 'spring or well' water compared to consumption of 'piped water'. On the other hand compared to 'not chlorinated water' 'spring or well' water was found to be protective when the individual washed hands adequately or used some type of latrine for defecation. The risk for rural dwellers was nearly 19 times the risk of urban dwellers for that water source.

3.5.9.2 Interactions for Ascaris infection on additive scale (Table 14)

When tested for interaction at the additive scale we found significant additive interaction between levels of the categories of our predictor variables. For *Ascaris*, the rural-urban status had a significant additive interaction with different levels of toilet facility. It had significant interaction when the toilet facility used was either 'some Latrine' versus 'septic tank or flush toilet' as well as when the toilet facility was either 'some latrine' versus 'Open defecation'. There was a significant interaction on the additive scale of rural-urban status with source of drinking water it was either 'spring or well' versus 'Not chlorinated water'.

3.5.9.3 Interactions for Trichuris infection on multiplicative scale (Table 13)

The independent variable that had most significant interactions at the multiplicative scale for *Trichuris* infection was used of shoes. Amongst non-geophagous individuals, use of open footwear or nothing was associated with more than four times the risk of infection than those who always wore closed footwear. Although *Trichuris* does not have dermo-penetrative infective forms, the potential for cutaneous contamination and mechanical transport of contaminated soil is minimized and thus accidental consumption of infective forms is avoided according protection and lower risk. For individuals with or without poor hand washing, the risk of infection is similarly higher in those who use open footwear or nothing. However, those who washed hands poorly showed lower risk compared to those who washed hands adequately. This could possibly be explained by the small sample size given the outcome was rare. Amongst resident of rural areas, identical shoe usage habits were associated with an eight times higher risk of infection. In individuals who wore closed footwear sometimes versus those who wore open footwear or nothing, a protective effect was seen for those were non-geophagous. This protective effect was evident if the individuals washed hands. Similarly, in rural residents, there was a protective effect seen with use of sometime use of closed footwear as compared to open footwear or bare-feet walking. This identifies an ordinal relationship in the pattern of use of footwear with highest risk of infection being associated with use of open footwear or bare-feet walking followed by a lower risk with use of closed footwear sometimes and lowest risk of infection with use of closed footwear all the time. This suggests that interventions aimed at improving footwear use patterns should promote use of closed footwear all the time or at least some of the time.

When the risk of infection was tested for rural-urban status in those who used closed footwear always, a protective effect was seen amongst rural residents compared to urban residents. Similarly, for use of open footwear or bare-feet walking, a protective effect was seen for rural residents compared to urban residents. A possible explanation for this could be the increased population density and greater unsanitary conditions with soil and earth contamination in the urban areas as compared to rural areas. This suggests that interventions targeted at improvement of use of footwear patterns would more likely be effective when targeted at urban residents. Also, rural residents were protected from infection compared to urban dwellers even when both used piped water as source of drinking water further supporting the effect of population density and unsanitary hygiene conditions in the urban areas.

In individuals who washed their hands adequately, use of ‘spring or well’ water for drinking posed a three and a half times greater risk of *Trichuris* infection compared to those who used piped water for drinking. For rural residents, similar drinking water sourcing was associated with a three times greater risk of infection. This again points out the possible lower quality of surface water sources – springs and wells due to their potential for contamination by sewage by rainfall runoff and human activities. In individuals who use a septic tank or flush toilets, the use of spring or well water for drinking resulted in a greater than four times risk on *Trichuris* infections. This shows that even though they had access to the highest possible type of improved sanitation, the use of contaminated surface water sources of springs or wells, nullified the protective effect of improved sanitation and further increased the risk of contamination greatly.

Amongst those who used some type of latrine for defecation, there a protective effect on those who resided in rural areas compared to the urban areas. Also, amongst rural residents, a protective effect was seen in those who used some type of latrine compared to a septic tank or flush toilet. This is counter-intuitive but can be explained by the poor coverage of sewage systems in rural areas suggesting that although there is access to septic tank and flush toilets, the disposal of fecal matter may not be efficient in inactivation of fecal pathogens or safe and adequate. Amongst rural residents a protective effect of use of some type of latrine was seen over open air defecation practice against *Trichuris* infection.

3.5.9.4 Interactions for Trichuris trichiura infection on additive scale (Table 16)

When tested for interactions amongst the independent variables on the additive scale, rural-urban status was found to interact significantly across levels of Use of shoes, type of toilet facility and source of drinking water. For use of shoes additive interaction was significant for 'Always closed shoes' versus 'sometimes closed shoes'. For type of toilet facility, additive interaction was significant for 'some latrine' versus 'septic tank or flush toilets'. For source of drinking water, the additive interaction was significant for 'Piped water +/- not chlorinated water' versus 'Packaged water', 'Piped water +/- not chlorinated water' versus 'Not chlorinated water' and 'spring or well' versus 'Packaged water'.

3.5.9.5 Interactions for Hookworm infection on multiplicative scale (Table 12)

There were very few significant interactions when tested for risk of hookworm infection. Individuals who did not wash their hands adequately were protected amongst those who were non-geophagous. This could possibly be due to sampling method used or the different mode of infection of hookworm that is dependent of dermo-penetrative larval forms. Also, individuals who washed hands poorly were protected amongst those who had access to piped water source for drinking water. Amongst urban residents, the use of 'spring or well water' as source of drinking water was associated with a fifteen times higher risk of infection with hookworm compared to those using piped water as source of drinking water. This could be due to the contamination of the well or spring and its surrounding areas along the edge with infective forms. Also, humans drawing water from these sources may not wear closed footwear during water collection increasing the contact between exposed bare feet in the contaminated soil around the wells and springs with greater risk of infection with hookworm larvae. Important to recognize here is the opportunity of targeted intervention for decreasing risk due to this cause is to build an embankment of cement around the water source to drain away spoilt water away from the water's edge and minimize constant with soil bearing infective larvae.

3.5.9.6 Interactions for Hookworm infection on additive scale (Table 15)

Poor hand washing was found to have significant interactions on the additive scale across different levels of source of drinking water when tested for infection with hookworm. The interactions were significant when source of drinking water was tested in the following pairs – 'Piped water +/-non-chlorinated water' versus 'packaged water', 'Piped water +/-non-chlorinated water' versus Not chlorinated water' and 'spring or well' versus 'Not chlorinated water'. Rural-urban status also displayed additive interaction across levels of source of drinking water. It was significant when levels of source of drinking water were 'Piped water +/- not chlorinated water' vs 'Not chlorinated water' and 'spring or well' vs 'Not chlorinated water'. Source of drinking water had significant additive interaction with the type of toilet facility when the source of drinking water 'spring or well 'or 'Not chlorinated water' when toilet facility was 'some latrine' or 'septic tank or Flush toilet'.

3.6 Conclusion and Recommendations

The prevalence of STHI in El Salvador is lower than has been previously reported. The countries neighboring El Salvador have a much higher prevalence of STHI in children. Honduras has 22.3% infection prevalence with *Ascaris*, 34 % infection prevalence with *Trichuris* and 0.9% infection prevalence with hookworm.[45] A study in eastern Guatemala done in school children found prevalence of 30% for hookworm, 52% for *Ascaris* and 39% for *Trichuris*.[46] Ecological zones have a very significant association with geohelminth infection prevalence. All the eco-epidemiological zones had higher odds of infection compared to the mountains region. Central depression region is associated with significant odds ratios for infection with *Ascaris* and *Trichuris* infections and interventions for prevention should be targeted in this region. This should be followed by targeted interventions for prevention of *Ascaris* in the volcanic chain regions and against *Trichuris* in the coastal chain region. Significant risk factors persist for the transmission of geohelminths like inadequate coverage by safe drinking water and safe sanitation. People should be educated to treat drinking water prior to consumption to minimize risk of infection. The preventive efforts should be targeted more at urban populations who do not have access to safe drinking water and sanitation as rural residence was protective against infection with *Trichuris* and hookworms. This is possibly due to higher population density and strengthening of urban sanitation and water supply infrastructure should be pursued in the urban areas. Increased coverage with safe sanitation and education to desist from open defecation should be stressed. In the rural areas, strengthening of the existing sanitation infrastructure by increasing the coverage of latrines and improving the efficiency and efficacy of the toilets in inactivation of fecal pathogens, such as using urea as an additive to feces, should be pursued as they are low resource measures. Also, promotion of use of closed footwear in favor of use of open footwear or bare-feet walking should be used as a strategy for geohelminth control.

Table 4. List of Interaction terms tested and rationale for analyses in Survey - Prevalence of STHI among school –age children in El Salvador, 2012

No.	Interaction pairs tested	Rationale
1	Geophagy with Use of shoes	Individuals engaging in geophagy less likely to use shoes
2	Rural-urban status with source of drinking water	Water source less likely to be piped water in rural regions
3	Rural-urban status with Toilet Facility	Open defecation is more common in rural areas
4	Poor hand washing with Use of Shoes	Individuals engaged in poor handwashing less likely to use shoes
5	Poor hand washing with source of drinking water	Poor handwashing is more likely in areas without piped water
6	Rural-urban status and Use of shoes	Children in urban regions more likely to use shoes
7	Source of drinking water and Toilet Facility	Interaction of Sanitation and water supply

Table 5. Distribution of study subjects by Sources of drinking water in Survey - Prevalence of STH among school –age children in El Salvador, 2012

Source of Drinking Water	Frequency	Percent
Piped potable water +/- non-chlorinated water	970	74.05
Packaged Water	35	2.67
Spring or well water	278	21.22
Non-chlorinated	27	2.06
TOTAL	1310	100

Table 6. Distribution of study subjects by Type of toilet facility in Survey - Prevalence of STH among school –age children in El Salvador, 2012

Toilet Facility	Frequency	Percent
Latrine or Composting latrine	409	31.22
Septic tank or Flush toilet	820	62.6
Open Air	81	6.18
TOTAL	1310	100

Table 7. p-values of chi-square tests for association between independent and dependent variables in analyses of data in Survey - Prevalence of STH among school –age children in El Salvador, 2012

Predictor Variable	**Outcome Variables**		
	***Ascaris* infection**	***Trichuris* infection**	**Hookworm infection**
Age	NS	NS	NS
Gender	NS	NS	NS
Eco-epidemiological Zone	0.0031	<0.001	NS
Urban-Rural Status	NS	0.0219	NS
Eating Street Food	NS	NS	NS
Geophagy	NS	NS	NS
Poor Hand Washing	NS	NS	0.0313
Use of Shoe	0.0035	<.0001	0.0336
Toilet Facility	0.0032	NS	NS
Source of drinking water	NS	0.0049	NS

NS=Not Significant

Table 8. Odds ratio estimates and 95% CI for unadjusted and fully adjusted logistic regression models for *Ascaris* infection in Survey - Prevalence of STH among school –age children in El Salvador, 2012

Predictor Variable	**Unadjusted**			**Full Adjustment**		
	Odds Ratio Point Estimate	**95% Wald Confidence Intervals**		**Odds Ratio Point Estimate**	**95% Wald Confidence Intervals**	
Age 9 vs 8	NS	NS	NS	NS	NS	NS
Age 10 vs 8	NS	NS	NS	NS	NS	NS
Eating Street Food 'Yes' vs 'No'	NS	NS	NS	NS	NS	NS
Eco-epidemiologic Zone 'Volcanic chain' vs 'Mountain'	NS	NS	NS	5.868	1.089	31.612
Eco-epidemiologic Zone 'Central Depression' vs 'Mountain'	7.348	1.673	32.281	10.500	2.244	49.123
Eco-epidemiologic Zone 'Coastal chain' vs 'Mountain'	NS	NS	NS	NS	NS	NS
Eco-epidemiologic Zone 'Coastal Plain' vs 'Mountain'	4.689	1.003	21.926	NS	NS	NS
Geophagy 'Yes' vs 'No'	NS	NS	NS	NS	NS	NS
Poor Handwashing 'Yes' vs 'No'	NS	NS	NS	NS	NS	NS
Gender 'Female' vs 'Male'	NS	NS	NS	NS	NS	NS
Use of Shoes 'sometimes Closed shoes' vs 'Always footwear'	NS	NS	NS	NS	NS	NS
Use of Shoes 'sandals or nothing' vs 'Always footwear'	2.473	1.061	5.765	2.582	1.051	6.342
Toilet Facility 'some Latrine' vs 'septic tank or Flush toilet'	NS	NS	NS	NS	NS	NS
Toilet Facility 'Open Air' vs 'septic tank or Flush toilet'	4.216	1.706	10.419	NS	NS	NS
Urban-Rural Status 'Rural' vs 'Urban'	NS	NS	NS	NS	NS	NS
Source of Drinking Water 'Packaged' vs 'Piped water +/- chlorination'	NS	NS	NS	NS	NS	NS
Source of Drinking Water 'spring or well Water' vs 'Piped water +/- chlorination'	2.519	1.256	5.055	3.199	1.377	7.430
Source of Drinking Water 'Not Chlorinated Water' vs 'Piped water +/- chlorination'	NS	NS	NS	NS	NS	NS

NS=Not Significant

Table 9. Odds ratio estimates and 95% CI for unadjusted logistic regression models for *Trichuris* infection in Survey - Prevalence of STH among school –age children in El Salvador, 2012

Predictor Variable	**Unadjusted**			**Full Adjustment**		
	Odds Ratio Point Estimate	**95% Wald Confidence Intervals**		**Odds Ratio Point Estimate**	**95% Wald Confidence Intervals**	
Age '8' vs '10'	NS	NS	NS	NS	NS	NS
Age '9' vs '10'	NS	NS	NS	NS	NS	NS
Eating Street Food 'Yes' vs 'No'	NS	NS	NS	NS	NS	NS
Eco-epidemiologic Zone 'Volcanic chain' vs 'Mountain'	NS	NS	NS	NS	NS	NS
Eco-epidemiologic Zone 'Central Depression' vs 'Mountain'	8.089	1.018	64.298	NS	NS	NS
Eco-epidemiologic Zone 'Coastal chain' vs 'Mountain'	8.361	1.052	66.465	NS	NS	NS
Eco-epidemiologic Zone 'Coastal Plain' vs 'Mountain'	33.121	4.475	245.133	31.946	4.142	246.415
Geophagy 'Yes' vs 'No'	NS	NS	NS	NS	NS	NS
Poor Handwashing 'Yes' vs 'No'	NS	NS	NS	NS	NS	NS
Gender 'Female' vs 'Male'	NS	NS	NS	NS	NS	NS
Use of Shoes 'sometimes Closed shoes' vs 'Always footwear'	NS	NS	NS	NS	NS	NS
Use of Shoes 'sandals or nothing' vs 'Always footwear'	4.595	2.086	10.122	4.297	1.827	10.105
Toilet Facility 'some Latrine' vs 'septic tank or Flush toilet'	NS	NS	NS	NS	NS	NS
Toilet Facility 'Open Air' vs 'septic tank or Flush toilet'	NS	NS	NS	NS	NS	NS
Urban-Rural Status 'Rural' vs 'Urban'	0.504	0.276	0.921	0.414	0.196	0.877
Source of Drinking Water 'Packaged' vs 'Piped water +/- chlorination'	NS	NS	NS	NS	NS	NS
Source of Drinking Water 'spring or well Water' vs 'Piped water +/- chlorination'	2.694	1.528	4.750	3.105	1.541	6.253
Source of Drinking Water 'Not Chlorinated Water' vs 'Piped water +/- chlorination'	NS	NS	NS	NS	NS	NS

NS=Not Significant

Table 10. Odds ratio estimates and 95% CI for unadjusted logistic regression models for hookworm infection in Survey - Prevalence of STH among school –age children in El Salvador, 2012

Predictor Variable	**Unadjusted**			**Full Adjustment**		
	Odds Ratio Point Estimate	**95% Wald Confidence Intervals**		**Odds Ratio Point Estimate**	**95% Wald Confidence Intervals**	
Age '8' vs '10'	NS	NS	NS	NS	NS	NS
Age '9' vs '10'	NS	NS	NS	NS	NS	NS
Eating Street Food 'Yes' vs 'No'	NS	NS	NS	NS	NS	NS
Eco-epidemiologic Zone 'Volcanic chain' vs 'Mountain'	NS	NS	NS	NS	NS	NS
Eco-epidemiologic Zone 'Central Depression' vs 'Mountain'	NS	NS	NS	NS	NS	NS
Eco-epidemiologic Zone 'Coastal chain' vs 'Mountain'	NS	NS	NS	NS	NS	NS
Eco-epidemiologic Zone 'Coastal Plain' vs 'Mountain'	NS	NS	NS	NS	NS	NS
Geophagy 'Yes' vs 'No'	NS	NS	NS	NS	NS	NS
Poor Handwashing 'Yes' vs 'No'	0.324	0.110	0.853	0.175	0.043	0.714
Gender 'Female' vs 'Male'	NS	NS	NS	NS	NS	NS
Use of Shoes 'sometimes Closed shoes' vs 'Always footwear'	9.188	1.209	69.849	8.647	1.117	66.962
Use of Shoes 'sandals or nothing' vs 'Always footwear'	8.697	1.082	69.903	NS	NS	NS
Toilet Facility 'some Latrine' vs 'septic tank or Flush toilet'	NS	NS	NS	NS	NS	NS
Toilet Facility 'Open Air' vs 'septic tank or Flush toilet'	NS	NS	NS	NS	NS	NS
Urban-Rural Status 'Rural' vs 'Urban'	NS	NS	NS	0.307	0.095	0.989
Source of Drinking Water 'Packaged' vs 'Piped water +/- chlorination'	NS	NS	NS	NS	NS	NS
Source of Drinking Water 'spring or well Water' vs 'Piped water +/- chlorination'	NS	NS	NS	NS	NS	NS
Source of Drinking Water 'Not Chlorinated Water' vs 'Piped water +/- chlorination'	NS	NS	NS	NS	NS	NS

NS=Not Significant

Table 11. Significant Multiplicative Interaction for the outcome *Ascaris* infection in Survey - Prevalence of STH among school –age children in El Salvador, 2012

Interaction term	**Odds ratio**		
	Estimate	**95%CI**	
Use of Shoe ‘sandals or nothing’ vs ‘Always Footwear’ at Geophagy = ’No’	2.940	1.155	7.481
Poor hand washing ‘Yes’ vs ‘No’ at Source of Drinking Water = ‘spring or well’	16.677	2.701	102.972
Source of Drinking Water ‘spring or well’ vs ‘Not Chlorinated’ at Poor hand washing= ‘No’	0.046	0.003	0.734
Source of Drinking Water ‘spring or well’ vs ‘Piped Water +/- not chlorinated water’ at Poor hand washing= ‘Yes’	28.862	6.717	124.020
Use of Shoes ‘sometimes Closed shoes’ vs ‘sandals or nothing’ at Rural-urban status = ‘Urban’	0.056	0.005	0.602
Source of Drinking Water ‘spring or well’ vs ‘Piped Water +/- Not –chlorinated water’ at Rural-urban status = ‘Urban’	38.393	4.396	335.331
Source of Drinking Water ‘spring or well’ vs ‘Piped Water +/- Not –chlorinated water’ at Rural-urban status = ‘Rural’	2.624	1.075	6.407
Source of Drinking Water ‘spring or well’ vs ‘Piped Water +/- Not –chlorinated water’ at Toilet Facility= ‘some Latrine’	7.796	2.059	29.521
Toilet Facility ‘some Latrine’ vs ‘Open Air’ at Source of Drinking Water = ’Pipe Water =/- not chlorinated water’	0.168	0.037	0.754
Toilet Facility ‘Open Air’ vs ‘septic Tank or Flush Toilet’ at Source of Drinking Water = ’Piped water =/-not chlorinated water’	4.931	1.227	19.815

Table 12. Significant Multiplicative Interaction for the outcome Hookworm infection in Survey - Prevalence of STH among school –age children in El Salvador, 2012

Interaction term	Odds ratio		
	Estimate	95%CI	
Use of Shoes ‘sometimes closed shoes’ vs ‘Always Footwear’ at Geophagy= ‘No’	8.063	1.038	62.630
Poor Hand washing ‘Yes’ vs ‘No’ at Source of drinking water = ‘Piped water +/- not chlorinated water’	0.163	0.028	0.947
Rural-urban status = ‘Rural’ vs ‘Urban’ at Source of drinking water = ‘spring or well’	0.038	0.003	0.574
Source of drinking water = ‘spring or well’ vs ‘Piped water +/- not chlorinated water’ at Rural-urban status = ‘Urban’	15.067	1.053	215.630

Table 13. Significant Multiplicative Interaction for the outcome *Trichuris* infection in Survey - Prevalence of STH among school –age children in El Salvador, 2012

Interaction term	Odds ratio		
	Estimate	95%CI	
Use of shoes ‘sandals or nothing’ vs ‘Always Footwear’ at Geophagy= ‘No’	4.371	1.862	10.259
Use of shoes ‘sometimes closed shoes’ vs ‘sandals or nothing’ at Geophagy= ‘No’	0.219	0.110	0.436
Use of shoes ‘sandals or nothing’ vs ‘Always Footwear’ at Poor hand washing= ‘No’	5.426	1.502	19.607
Use of shoes ‘sandals or nothing’ vs ‘Always Footwear’ at Poor hand washing= ‘Yes’	3.775	1.137	12.532
Use of shoes ‘sometimes closed shoes’ vs ‘sandals or nothing’ at Poor hand washing= ‘Yes’	0.169	0.047	0.611
Source of drinking water ‘spring or well’ vs ‘Piped water +/- not chlorinated water’ at Poor hand washing= ‘No’	3.615	1.556	8.401
Use of shoes ‘sandals or nothing’ vs ’Always Footwear’ at Rural-urban status = ‘Rural’	8.225	2.289	29.554
Use of shoes ‘sometimes closed shoes’ vs ’Sandals or Nothing ’ at Rural-urban status = ‘Rural’	0.402	0.189	0.853
Rural-urban status ‘Yes’ vs ‘No’ at Use of shoes= ‘Always Footwear’	0.058	0.011	0.303
Rural-urban status ‘Yes’ vs ‘No’ at Use of shoes= ’Sandals or Nothing ’	0.248	0.090	0.679
Rural-urban status ‘Yes’ vs ‘No’ at Toilet Facility = ‘some Latrine’	0.096	0.028	0.334
Toilet Facility ‘some Latrine’ vs ‘septic tank or Flush toilet’ at Rural-urban status = ‘Rural’	0.376	0.155	0.915
Toilet Facility ‘some Latrine’ vs ‘Open Air’ at Rural-urban status = ‘Rural’	0.256	0.071	0.926
Rural-urban status ‘Yes’ vs ‘No’ at Source of drinking water= ‘Piped water +/- not chlorinated water’	0.425	0.187	0.968
Source of drinking water ‘spring or well’ vs ‘Piped water +/- not chlorinated water’ at Rural-urban status = ‘Rural’	3.016	1.425	6.384
Source of drinking water ‘spring or well’ vs ‘Piped water +/- not chlorinated water’ at Toilet Facility= ‘septic tank or Flush toilet’	4.336	1.802	10.435

Table 14. Significant RERI values for test of interaction on the Additive Scale for *Ascaris* infection in Survey - Prevalence of STH among school –age children in El Salvador, 2012

Interaction Term	RERI		
	Estimate	95% CI	
Rural-urban status 'Yes' vs 'No' when toilet facility is 'some Latrine' vs 'septic tank or Flush Toilet'	0.87067	0.57895	1.16240
Rural-urban status 'Yes' vs 'No' when toilet facility is 'some Latrine' vs 'Open Air'	2.17290	0.10532	4.2408
Rural-urban status 'Yes' vs 'No' when source of drinking water is 'Spring or well' vs 'Not Chlorinated water'	0.98832	0.89783	1.07882

Table 15. Significant RERI values for test of interaction on the Additive Scale for Hookworm infection in Survey - Prevalence of STH among school –age children in El Salvador, 2012

Interaction Term	RERI		
	Estimate	95% CI	
Poor hand washing 'Yes' vs 'No' when source of drinking water is 'Piped water +/- not chlorinated water' vs 'Packaged water'	0.79006	0.40012	1.18000
Poor hand washing 'Yes' vs 'No' when source of drinking water is 'Piped water +/- not chlorinated water' and 'Not chlorinated water'	0.79006	0.39981	1.18030
Poor hand washing 'Yes' vs 'No' when source of drinking water is Spring or well' vs 'Not chlorinated water'	0.83262	0.48417	1.18106
Rural-urban status 'Yes' vs 'No' when source of drinking water is 'Piped water +/- not chlorinated water' vs 'Not chlorinated water'	0.52808	0.010708	1.04545
Rural-urban status 'Yes' vs 'No' when source of drinking water is Spring or well' vs 'Not chlorinated water'	0.96706	0.87080	1.06333
Source of drinking water 'spring or well' vs 'Not chlorinated water ' when toilet facility is 'some latrine' vs Septic tank or flush toilet'	0.79204	0.23738	1.34670

Table 16. Significant RERI values for test of interaction on the Additive Scale for *Trichuris* infection in Survey - Prevalence of STH among school –age children in El Salvador, 2012

Interaction Term	RERI		
	Estimate	95% CI	
Rural-urban status 'Yes' vs 'No' when Use of shoes is 'Always closed shoes' vs 'sometimes closed shoes'	1.15137	0.93290	1.36984
Rural-urban status 'Yes' vs 'No' when toilet facility is 'some Latrine' vs 'septic tank or Flush Toilet'	0.88892	0.66346	1.11437
Rural-urban status 'Yes' vs 'No' when source of drinking water is 'Piped water +/- not chlorinated water' vs 'Packaged water'	1.07376	0.19414	1.95338
Rural-urban status 'Yes' vs 'No' when source of drinking water is 'Piped water +/- not chlorinated water' vs 'Not chlorinated water'	1.15331	0.04115	2.26521
Rural-urban status 'Yes' vs 'No' when source of drinking water is 'spring or well' vs 'Packaged water'	0.86182	0.53242	1.19121

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CHAPTER 4:

PIPED WATER SUPPLIER OF DRINKING WATER AND SOIL TRANSMITTED HELMINTH INFECTIONS IN SCHOOL CHILDREN IN EL SALVADOR

4.1 Abstract

4.1.1 Background

Piped water supply in El Salvador for drinking is through various different types of suppliers. They are ‘Adminstracion Nacional de Acueductos y Alcatarillodos’ (ANDA), Decentralized operators under contract management and those not under contract management. Lack of safe water supply for drinking is one of the key factors for soil transmitted helminth infection. We looked at the risk of infection in the areas served by the three types of suppliers of piped drinking water.

4.1.2 Methods

Data of 1310 children aged 8-10 years was analysed after identifying the type of supplier of piped water in their municipality. Three categorical variables were created to assess the possibility of increased risk based on the type of piped water supplier. One variable had two categories for ‘ANDA’ and ‘non-ANDA’ suppliers of piped water. The second had all three categories of suppliers while the third one had only the two types of decentralized operators. Chi-square test was done to identify significant associations. Logistic regression was done to determine the odds ratios for risk of infection with *Ascaris lumbricoides*, *Trichuris trichiura* and hookworms.

4.1.3 Results

All the three variables created for the type of supplier of piped drinking water were significantly associated with *Ascaris* infections. ANDA vs non-ANDA (p=0.0085); ANDA vs decentralized operator under contract management vs decentralized operator not under contract management (p=0.0025); and decentralized operator under contract management vs decentralized operator not under contract management (p=0.0013). No significant association was found for infection with *Trichuris trichiura* and hookworm. Odds Ratios were identified for *Ascaris* infection as follows. Non-ANDA compared to ANDA [OR 2.78 (1.257-6.146)], decentralized operators under contract management compared to ANDA [4.072 (1.722-9.631)] and decentralized operators under contract management when compared to those not under contract management [2.879 (1.472-5.631)]

4.2 Introduction:

Piped water supply in El Salvador is provided primarily by the 'Administracion Nacional de Acueductos y Alcantarillodos' (ANDA) agency in EL Salvador. Aside from ANDA decentralized operators also are engaged in provision of piped water to some areas of El Salvador. Amongst these Decentralized operators there are two types. The first ones are those that are under contract for provision of piped water and the second are those that not under contract for provision of piped water. The quality of piped water is supposed to be the best quality and fit for drinking 'agua potable'. These decentralized operators are what are known as the Small Scale Infrastructure Providers (SSIP). They provide coverage of piped water services to regions that are not covered by ANDA. However, the SSIP are known to have lower standards of functioning and operations than ANDA to save costs and provide affordable services to the communitive they serve.[1] Also, in rural areas, associations of community members collect contributions to purify water and make it safe for drinking prior to supply through pipe system infrastructure. However, it is not uncommon for these collections to be inadequate to purchase adequate amounts of chemical reactants to treat the water and make it safe for drinking prior to distribution through the piped systems.[2] This raises the possibility the quality of piped water supplied being substandard thereby posing health risks to the communities using piped water from these SSIP and associations for drinking purposes.

4.3 Methods

We used the data collected by the Ministry of Health (MOHES) and Pan America Health Organization in the Survey of soil transmitted helminths in school children in El Salvador. The details of data collection are described elsewhere.[3] After data cleaning as elaborated in chapter 3, we identified municipalities that received piped water supply by ANDA and decentralized operators. Based on the '2011 Boletin Estadistica' published by the 'Administracion Nacional de Acueductos y Alcantarillados' in El Salvador three new variables PWS1-3 were created.[4] Data from 1310 school children aged 8-10 years was used in this analysis.

The three variables created were as follows-a) PWS1 was created to determine if there is an association of outcome with ANDA and Non-ANDA piped water supply, b) PWS2 was created to determine if there is an association of outcome with ANDA, decentralized operators under contract management and those not under contract management, and c) PWS3 was created to determine if there is an association of outcome with decentralized operators under contract management and those under contract management. The outcomes that we tested were infection with *Ascaris lumbricoides*, infection with *Trichuris trichiura* and infection with hookworms. For determination of significant association chi-square test was used. Logistic regression was used to determine specific odds ratios risk of outcome.

4.4 Results

Chi-square tests results for *Ascaris* infection, *Trichuris trichiura* infection and hookworm infection are shown in Table 17. The odds ratios for the risk of *Ascaris* infection, *Trichuris trichiura* infection and hookworm infection for each of the types of piped water supplier of drinking water is given in Table 18, Table 19 and Table 20 respectively.

4.5 Discussion

Our results showed a significant difference in the association of infection with *Ascaris lumbricoides* amongst the three different supplier of piped water for drinking. Compared to ANDA, individuals who resided in areas supplied by the decentralized operators under contract management were 4 times more likely to be infected with *Ascaris lumbricoides*. While amongst individuals who resided in areas receiving drinking water through piped supply by decentralized operators under contract management, the risk of infection was 2.8 times higher than amongst those who resided in areas receiving drinking water through piped supply by decentralize operators not under contract management.

Soil transmitted helminth infections are known to persist in conditions of unsafe sanitation and drinking water supply. Piped water supply is considered to be free of infective pathogens and thus safe for drinking. However, breaches of continuity in the supply system create the potential for contamination of treated water. Fecal contamination of this water can lead to spread of water-borne illnesses. Our results show that there is a significant association between the supplier of piped water for drinking and *Ascaris* infection. We did not find any significant association with *Trichuris trichiura* infection or hookworm infection. This difference could possibly be due to different levels of hardiness of the infective forms of the three types of parasites. *Ascaris* ova are hardy organisms and are thus used in research to determine the most effective method for inactivation of fecal pathogens and also water quality testing.[5] *Trichuris* is less resistant to inactivation and hookworm ova are most fragile and easily inactivated in the environment. ANDA is the National administration for water and sanitation. Being a central agency it has better quality control measures in place and a much greater population of service compared to the decentralized operators. The decentralized operators fill in the needs of the people residing in areas not serviced by ANDA. These entities operate at a much smaller scale and charge a monetary price for their services. Most of their clientele is in the underserved and rural areas. This being so, their operating budgets are smaller and quality of service and operation of a lower standard than ANDA.[1] This could potentially affect the quality of water that they supply through the pipe system for drinking. McKnight in his 2014 doctoral research identified varying standards of water quality sourced from improved sources of water i.e. piped water in Costa Rica.[6] He studied the water quality in communities of Paraiso, Venado, La Florida and San Jose de la Montana. He found that 61% of the household water samples failed to pass the WHO standards for total fecal coliform count by going over the single standard limit of zero. Most of these water sources were wells from which water was piped into the houses in communities. By WHO standards, household connection of piped water is considered as an improved water source.[7] However, the improvement is only in terms of the engineering construct of the physical infrastructure, but not from a microbiological perspective. WHO defines safe drinking water as meets or exceeds the standards of safe water by WHO or national standards for chemical, microbial and physical qualities.[7] Bain et al in their 2014 systematic review on fecal contamination of drinking water in low and middle income countries concluded in general unimproved water sources imposed greater risk of infection with fecal pathogens compared to improved water sources. In their review they found that in 38% of the studies (n=191), fecal infective bacterial levels in water samples from improved sources exceeded the WHO recommended levels. They caution that an overestimation of access to safe water results from assuming that 'improved water source' to be the same as 'safe water'.[8] This leads to inflated levels of safe water access thereby wrongly indicating progress towards the Millennium Development Goals (MDG).

4.6 Conclusions

The quality of water supplied by the different suppliers for drinking purposes seems to lack a uniform standard demonstrating a statistically significant association for infection with *Ascaris lumbricoides* as well as an increased risk. Further investigation into the quality of water supplied by the decentralized operators under contract management is recommended. Uniform standard operating procedures must be developed and implemented for ANDA and decentralized operators supplying piped drinking water to ensure uniform quality and safety of drinking water supply to all people served and control the number of soil transmitted helminth infections.

Table 17. Chi-square test showing significant association of infection with different types of soil transmitted helminthes and different types of suppliers of piped water for drinking use in Survey - Prevalence of STH among school –age children in El Salvador, 2012

	Ascaris infection (p-value)	*Trichuris trichiura* infection (p-value)	Hookworm infection (p-value)
ANDA versus Decentralized operators as suppliers of piped water	0.0085	0.9060	0. 5389
ANDA versus Decentralized operators under contract versus Decentralized operator not under contract as suppliers of piped water	0.0025	0.5414	0.4288
Decentralized operators under contract versus Decentralized operator not under contract as suppliers of piped water	0.0013	0.3046	0.1932

Table 18. Odds Ratios for the risk of *Ascaris* infection each of the types of piped water supplier of drinking water in Survey - Prevalence of STH among school –age children in El Salvador, 2012

	Ascaris infection		
	Odds Ratio	95% CI	
Decentralized operators as suppliers of piped water versus ANDA (ref)	2.780	1.257	6.146
Decentralized operators under contract management as suppliers of drinking water versus ANDA (ref)	4.072	1.722	9.631
Decentralized operator not under contract management as suppliers of piped water versus ANDA (ref)	1.954	0.792	4.822
Decentralized operators under contract management as suppliers of drinking water versus Decentralized operator not under contract as suppliers of piped water (ref)	2.879	1.472	5.631

Table 19. Odds Ratios for the risk of *Trichuris trichiura* infection for each of the types of piped water supplier of drinking water in Survey - Prevalence of STH among school –age children in El Salvador, 2012

	Trichuris trichiura infection		
	Odds Ratio	95% CI	
Decentralized operators as suppliers of piped water versus ANDA (ref)	0.967	0.559	1.674
Decentralized operators under contract management as suppliers of drinking water versus ANDA (ref)	0.724	0.332	1.578
Decentralized operator not under contract management as suppliers of piped water versus ANDA (ref)	1.131	0.621	2.059
Decentralized operators under contract management as suppliers of drinking water versus Decentralized operator not under contract as suppliers of piped water (ref)	0.685	0.331	1.417

Table 20. Odds Ratios for the risk of hookworm infection for each of the types of piped water supplier of drinking water in Survey - Prevalence of STH among school –age children in El Salvador, 2012

	Hookworm infection		
	Odds Ratio	95% CI	
Decentralized operators as suppliers of piped water versus ANDA (ref)	1.298	0.564	2.987
Decentralized operators under contract management as suppliers of drinking water versus ANDA (ref)	1.756	0.670	4.6
Decentralized operator not under contract management as suppliers of piped water versus ANDA (ref)	1.0	0.369	2.706
Decentralized operators under contract management as suppliers of drinking water versus Decentralized operator not under contract as suppliers of piped water (ref)	1.756	0.744	4.145

4.7 Reference

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CHAPTER 5:

A NOVEL STRATEGY FOR ENVIRONMENTAL CONTROL OF SOIL TRANSMITTED HELMINTHES

Note to Reader

This chapter has been previously published (Sharad, S. M., & Ricardo, I. (2012). A novel strategy for environmental control of soil transmitted helminthes. *Health Environ J, 3*(3), 28.) and is utilized with permission from the publisher.

5.1 Abstract

Soil transmitted helminthes (STH) are important neglected tropical diseases widespread in developing countries. Repeated administration of anti-helminthics to adults and children for treatment and prevention of reinfection is frequently needed. The study aims to determine the effectiveness of urea for the inactivation of *Ascaris suum* eggs in the feces collection chamber of dry toilets in a tropical developing country. Sixty samples of approximately 10,000 *Ascaris suum* eggs each were randomly distributed equally in two groups; urea treatment and urea non-treatment. The treatment group toilets had urea added to the feces. The eggs were harvested at days 1,2,3,4 and 5 and then processed for assessment of viability by incubation in 0.1N sulphuric acid for three weeks. Viability was confirmed by observation of the larval form inside the egg by microscopy. Parameters like duration of treatment, concentration of gaseous ammonia generated, peak temperature achieved and change in moisture level were assessed utilizing multiple linear regression. Duration of treatment for at least 72 h ($p<0.001$; $\alpha=0.05$) and gaseous ammonia concentrations of at least 109.5 ppm ($p<0.001$; $\alpha=0.05$) were found to have a statistically significant association with at least 50% inactivation rate for the *Ascaris suum* eggs in 33% of the samples. Moisture level change and the peak temperature did not show any statistically significant effect on the inactivation of *Ascaris suum* eggs in our study. Urea is a potential field agent for the inactivation of geohelminth eggs for environmental control of soil transmitted helminthes through treatment of feces. Conclusion: Urea is a potential field agent for the inactivation of geohelminth eggs for environmental control of soil transmitted helminthes through treatment of feces.

Keywords: soil transmitted, helminthes, environmental control, inactivation, urea

5.2 Introduction

Soil transmitted helminthes (STH) are important neglected tropical diseases widespread in developing countries. Repeated administration of anti-helminthics to adults and children for treatment and prevention of reinfection is frequently needed.[1] A variety of non-conventional decentralized sanitation technologies have been implemented in rural areas of developing countries with the objective of meeting the Millennium Development Goals.[2] Many of these technologies have been designed with sustainability aspects, such as the absence of water in the operation system, but are failing in others, including the protection and promotion of human health by securing adequate treatment of fecal material before being reused. This is mainly caused by the presence of very resistant micro-organisms, worm eggs, which survive very harsh environmental conditions.[3] Treatment of the fecal material with urea offers the opportunity of achieving total inactivation of fecal pathogens, including worm eggs, in a variety of physical and chemical conditions currently found in non-conventional sanitation technologies.[4, 5] This treatment option will advance the evolution of sustainable sanitation technologies by offering a solution to break the transmission of fecal pathogens in the absence of conventional wastewater treatment options. In addition, it will minimize the use of non-renewable resources and provide the option of recycling the sanitized fecal material as a soil conditioner/fertilizer in crops.

We studied the use of urea to sanitize fecal material in a specific non-conventional sanitation technology: the solar toilet. This sanitation technology developed in El Salvador is a centralized dry sanitation system (urine diversion) that has already met two important sustainability aspects, socio-cultural and institutional acceptance, and has proven to reduce enteric diseases among communities using this technology. [6] The preliminary aims of this pilot project were to determine if ammonia can effectively inactivate *Ascaris suum* eggs in the solar toilets during a fixed short duration of storage time required for inactivation of pathogens in the toilets and the variations of temperature, pH and moisture content in the feces amended with urea. Solar latrines have been previously studied and found to have the overall least prevalence of intestinal parasite infestation.[7] Ammonia has previously been shown to be effective in the inactivation of *Ascaris suum* eggs in the laboratory.[8] Nordin et al studied the efficacy of ammonia for inactivation of *Ascaris* eggs in source separated urine and feces at ambient temperatures. [9] This study was a pilot to evaluate the practical field usage of urea as an additive for inactivation of helminthic ova in the feces in solar toilets to further improve their efficacy at reducing infective helminthic ova.

5.3 Materials and Methods

Twenty toilets were selected for the preliminary experiments (Fig 10). The solar toilets were all Type IV. The selected toilets were distributed in two groups: one with the intervention (urea) and the other without the intervention. Nylon bags with *Ascaris suum* eggs were placed in aluminum bags created with aluminum mesh. Then, the toilet chambers were closed for treatment and opened at different times for collection of aluminum bags: 1, 2, 3, 4, and 5 days after treatment. The nylon bags removed were incubated according to USEPA standards and *Ascaris suum* viability was determined by standard microscopy. The time of exposure of toilets to the intervention (urea vs. no urea) was called “treatment period”. Thus, there were two toilets with the intervention and two toilets without the intervention that were harvested on each of days 1 through 5.

During the treatment period, temperature was measured constantly. Moisture, pH and ammonia content were measured before and after treatment period. Five toilets were randomly selected and an additional bag with fecal material implanted with *Ascaris* eggs in nylon and wire mesh baskets were placed outside the toilet under environmental conditions to see if there was any difference in the outcome depending on the placement of the feces containing Husky bag. The same measurements were carried out on these bags as well.

The experiments were conducted in El Salvador in 2011. On Day 0, the parasite eggs and intervention were placed in the chambers. From Day 1 to Day 5, the toilets were opened and aluminum bags were retrieved for processing and incubation of *Ascaris suum* eggs. The duration of treatment was 24 hours for eggs harvested on Day1, 48 hours for Day 2, 72 hours for Day 3, (6 hours for Day 4 and 120 hours for Day5. Viability of the eggs was determined 21 days after extraction and incubation.

5.3.1 Selection of Community

The community selected to participate in the preliminary evaluation was El Angel, Majahual, Departamento La Libertad, El Salvador. There are 25 solar toilets prototype IV built in El Angel community. A short evaluation about the operation and maintenance of each toilet was done. The toilets that were adequately operated and maintained will be selected. The inclusion criteria are described in Box 1.

5.3.2 Evaluation of Chemical and Physical Parameters

Toilets selected to participate in the preliminary experiments were evaluated for the moisture content and pH levels. Moisture content was ideally in the range of 23% and 50% and pH level from 8.0 to 10.0. Modification to both parameters was done if their values are out of these ranges. If parameters are out of range, then they were managed according to the schema shown in Box 2.

5.3.3 Preparation of the *Ascaris suum* Eggs

Ascaris suum eggs bought from Excelsior Sentinel and stored at 4°C were placed in nylon bags. The bags were created using nylon mesh with a pore size of 30 μm; this allowed interaction with the environment while preventing the loss of eggs from the bag. Each bag had approximately 10,000 eggs and they were kept in refrigeration at 4°C in deionized (DI) water until distributed in aluminum bags and placed in the toilet chambers. The viability of the *Ascaris suum* eggs from the batch to be used in the experiments was calculated according to USEPA standards.

The use of nylon bags avoided the dispersion of the *Ascaris suum* eggs in the fecal material and to facilitate the collection of the eggs from the solar toilets when sampling. Fertilized *Ascaris suum* eggs are 45 µm to 75 µm long by 35 µm to 50 µm wide; unfertilized eggs measure 88 µm to 94 µm long to 44 µm wide [10]. These nylon bags were designed and made by us in the laboratory. Under a Biosafety hood the *Ascaris* eggs were placed into the nylon bags and the nylon bags were then sealed using a heat sealer. The concentration of the *Ascaris* eggs suspension was 50,000/ml. 200µl of the suspension was placed in the nylon bags i.e. 10,000 eggs in each nylon bag. Each of the nylon bags was then placed in an aluminum wire mesh bag. The aluminum wire mesh bags were for protection of the nylon bag from physical damage during placement, storage and harvest from the feces bags in the community. These bags were then stored at 4^0 C in DI water until placement in the feces bags in community. At the time of placement the bags were labeled with code specific for its location, duration of treatment and intervention. At the time of harvest the aluminum bags were collected and placed in Ziploc bags.

5.3.4 Urea Addition

Urea was added to the fecal material according to weight. The concentration of urea that was used was 1% weight/weight (1 gram of urea for each 99 grams of fecal material). The fecal material from the solar toilet was measured to a weight of 20 pounds using an on field balance that was suspended and set aside. Then, 0.2 pound of urea was added to the black Husky bag and the fecal material was transferred using a shovel and adding the *Ascaris suum* nylon bags during the process. The Husky bag was then sealed using duct tape. The sealed plastic bag was left inside the chamber for the treatment period. A similar bag was placed in the external environment outside five randomly selected toilets. Each solar toilet was sampled once to eliminate confounding of results due to loss of ammonia experienced when the bag was opened to remove the nylon bag with the parasite eggs. For control samples, the same procedure was followed but no urea was added. Due diligence was practiced to avoid contamination of control samples with urea or urea treated fecal material.

The following physical and chemical parameters were measured. The temperature inside the chamber was registered with a temperature data logger (Track-It Temperature Data Logger, MicroDAQ). This device was placed inside each Husky bag. For protection, the logger was covered by double Ziploc bags. Moisture and pH of fecal material was measured before the intervention and after the treatment period. The first measurement was done before placing urea in the bag for solar toilets. Second measurement was done at the time of *Ascaris suum* aluminum bags collection from the toilets. A soil pH and moisture meter was used to monitor this parameter. Ammonia gas concentration was measured prior to harvest of the *Ascaris suum* eggs containing nylon bags in the aluminum wire mesh bag. The measurement was done by the MiniRAE 3000 meter for measurement of gases and volatile substances. Two *Ascaris suum* bags were collected from each Husky bag. The time at which these bags were removed from each Husky bag varied. The first collection took place after 24 hours of treatment; the second collection took place after 48 hours; the third collection after 72 hours; the fourth collection after 96 hours and finally the last collection took place after 120 hours of treatment. Every time a Husky bag was opened for collection of bags, ammonia gas concentration, moisture and pH of fecal material were measured and registered. Each nylon bag removed was placed in a Ziploc bag for transportation to the lab facilities. In the lab the bags were washed in DI water and transferred to tubes containing 0.1N sulfuric acid for incubation at 28^0C for three weeks. The tubes were not closed to be air-tight to allow oxygen availability to the eggs. The level of sulfuric acid was monitored every two days and top up with DI water done to ensure adequate acid concentration and avoid desiccation of the eggs. After 21 days of incubation the nylon bags were removed from the tubes and the *Ascaris suum* eggs were examined for viability under the microscope at 40X and 100X magnification. 400 eggs were counted for each sample. EPA standards and protocols for disposal of bio-hazardous material were followed for disposal of the *Ascaris* eggs and contaminated materials after the evaluation for viability.

The data was analyzed using the SAS v9.2 software for statistical analysis. Alpha value was set at 0.05 for the analysis.

5.4 Results

Data was collected for 60 samples. Of these only 54 samples were included in the final analysis. Six of the samples belonging to three bags were not included due to failure of the temperature recording device. The preliminary univariate analysis indicates that for all samples irrespective of the intervention, the mean adjusted inactivation rate was 49.43 % (*sd 32.31, range 0 to 100*) with half the samples having an adjusted inactivation rate greater than 44%. The mean concentration of gaseous ammonia measured in the feces bags was 620.54 ppm (*sd 621.15, range 47.8 to 1832*) with half the samples having an exposure to at least 284 ppm of gaseous ammonia generated. The average peak temperature recorded was 36.78^{0}C (*sd 2.82, range 32.25 to 47*). Further Frequency analysis and Chi-square testing was done to determine the level of significance of the observed results. The ammonia gas level was divided into two levels of greater than or equal to 109.5 ppm and less than 109.5 ppm. The duration of treatment was divided into two strata of 72 hours or more and less 72 hours. The adjusted inactivation rate was divided in to two levels of 50% or higher and less that 50 %. The maximum temperature achieved was also divided into greater or equal to 36.78 ^{0}C and less than 36.78 ^{0}C. Frequency analysis showed that 40.74% samples ($n=22$) had an adjusted inactivation of 50% or higher and that 77.78% of the samples ($n=42$) were exposed to at least 109.5 ppm of gaseous ammonia generated. Of those that were exposed to the higher level of ammonia gas, 38.89% (n=21) had an adjusted inactivation rate of 50% or higher. Of the samples exposed to 109.5 ppm or lower level of ammonia gas, 1.85% (n=1) had an adjusted inactivation rate 50% or higher while 20.37% ($n=11$) had adjusted inactivation rate of less than 50% ($p<0.05$). 74.07% of the samples ($n=40$) had a treatment time of 72 hours or higher. An equal proportion 37.04 % (n=20) of samples had an adjusted inactivation of greater than or equal to 50% and less than 50%. 25.93% ($n=14$) had a treatment period of less than 72 hours of which only 3.70% ($n=2$) had inactivation of 50 % or higher and 22.22% ($n=12$) had adjusted inactivation rate of less than 50% ($p<0.05$). 40.74% of samples (n=22) achieved a peak temperature of 36.78 ^{0}C or higher. Of these 12.96% (n=12) had adjusted inactivation rate of 50% or higher while 27.78% ($n=15$) had adjusted inactivation rates less than 50%. 59.26 % of samples ($n=32$) achieved a peak temperature of less than 36.78^{0}C. Of these, 27.78% ($n=15$) had an adjusted inactivation rate of 50% or higher, while 31.48% ($n=17$) had adjusted inactivation rate of less than 50% ($p>0.05$). Linear regression analysis was done to determine the association between the ammonia gas concentrations, duration of treatment. Using the ammonia gas generated as the predictor variable and the adjusted inactivation rate as the outcome variable, the parameter estimate for the predictor variable was 0.01811 with a p-value of *0.0099*. This showed a weak though positive association between the concentration of ammonia and the adjusted inactivation rate. Similar analysis with the duration of treatment and adjusted inactivation rate gave the value of the parameter estimate as 7.048 with a p- value of *0.0299*. This showed a strong positive association between the adjusted inactivation rate and the duration of treatment. When both ammonia gas concentration and duration of treatment were used as predictor variables to generate a regression model for the adjusted inactivation rate, the parameter estimates were 0.024 and 9.94 with p-values of <0.0001 for either respectively. The parameter estimates show an increase when the two predictor variables are considered together suggesting greater positive association of duration of treatment with the adjusted inactivation rate. Collinearity testing did not show any significant collinearity. When the mean of proportionate viable eggs at each time of harvest was plotted against the duration of treatment, it showed a downward trend indicting progressive decrease in viability over the treatment period. This is seen in Figure. 2.

5.5 Discussion

Ascaris eggs have been known to survive wastewater and sludge disinfection procedures and harsh environmental conditions for many years[11, 12] and have therefore been designated as an index of hygiene quality.[12] Research into the use of ammonia for inactivation of *Ascaris* exists since the 1970's.

Chefranova observed inactivation of *Ascaris suum* eggs in solid and biological wastes at 18-22^0 C in less than 15 days.[13] Since 1990's the literature on the use of ammonia for inactivation of helminthic eggs has increased with most of the researchers working with sludge and ammonia solution and few have tested the use of ammonia in solid feces.[9]

Urea is a fertilizer that is widely used in agriculture as a source of nitrogen. It is cheap and easily available. It contains 46% nitrogen and easily hydrolyzed in the soil by urease to ammonium and bicarbonate ions.[14, 15] Significant amounts of ammonia are lost to the air due to volatilization after hydrolysis of urea in the soil.[14] There are various factors that affect the hydrolysis of urea and the rate of ammonia volatilization. They include temperature[16], presence of other chemicals,[17] concentration of urea itself[15] and pH.[15, 18] Most of the studies have measured and used dissolved ammonia as the inactivating agent for the inactivation of *Ascaris suum* eggs. [9, 19, 20]However, there are studies that have demonstrated that ammonia gas/fumes also inactivate *Ascaris spp*. Inactivation.[21] In this study we measured the concentration of ammonia gas in each Husky bag that held feces at the time of harvest with care taken to avoid leakage into the environment prior to measurement. This ammonia gas collected in the headspace in each bag during the treatment time. We did not measure the dissolved ammonia in the feces for practicality of on-site measurement. The pre-treatment concentration of ammonia gas was the same as the environment (5ppm, data not shown). The exact mechanism of action of ammonia for inactivation of the helminthic eggs is still not very clear. It has been suggested that increased temperature increases the permeability of the lipid membrane of the egg- shell allowing the un-ionized ammonia entry inside and disturbing the intra-ovular pH balance and effecting inactivation.[5, 8] We observed similar findings in our other lab studies of lower ammonia requirements with higher incubation temperatures (unpublished data). Ammonia has been shown to inactivate ova of other less tolerant parasites as well and include *Trichuris spp., Hymenolepis spp., Diphyllobothrium latum* and *Trichocephalus muris*.[22-24]

Temperature by itself is known to inactivate *Ascaris* eggs; however the temperatures required are much higher than the actual temperature peak achieved in the toilet chamber in the communities.[6] [25] The inconsistency in the peak temperature achieved is due to various factors such as the orientation of the solar panel of the toilet chamber, the shade of nearby trees and structures that preclude adequate sun exposure and thereby heating. Also, the people sometimes place rags and stones over the cover of the chamber to ensure adequate closure and avoid stench. Adding Ammonia seems to decrease the need for the temperature required for *Ascaris* inactivation. Ninety-nine percent inactivation of *Ascaris suum* eggs was observed in 3 days by Pecson et al by the addition of ammonia(5500ppm as N) to an aqueous solution at 34 ^{0}C and pH 11. [20]Nordin et al also found shorter inactivation times for *Ascaris suum* eggs with ammonia using urea as additive in fecal material in their studies.[9]. Our analysis did not show any statistically significant effect of the peak temperature achieved. Our findings of *Ascaris suum* eggs inactivation are intermediate between the findings of Moe et al that studied inactivation without any additives in the toilets in the community and Nordin et al that studied the inactivation of *Ascaris suum* eggs in feces with urea as additive in the laboratory. We set the temperature data loggers to record temperature every 15 seconds and thus could capture the highest peak temperature achieved. However, reaching the inactivating peak temperature does not translate into inactivation unless it is sustained as in the laboratory studies. [4] The lack of perfect control over temperature as well as a small sample size could possibly be responsible for our results. However, nonetheless, the study shows the practical applicability of use of urea an as additive as a strategic intervention to shorten and enhance the inactivation of ova of soil transmitted helminthes in the solar toilets used in the community. In this pilot study, we did not analyze the effect of pH or moisture since we controlled for them in the design of the study based on information presented by Nordin et al. Knowledge exists on the effect of pH on the inactivation of *Ascaris suum* ova.[5, 19]

Storage of fecal material itself under certain conditions can lead to inactivation of pathogens with periods of storage required up to 2-3 years or more depending on the conditions of temperature and moisture content of the fecal material. Lower moisture and higher temperature lead to faster inactivation process.[6, 12, 26, 27]

Cruz studied the inactivation of *Ascaris suum* eggs in the laboratory by replicating the physical and chemical conditions found in solar toilets. That study found that inactivation of *Ascaris spp*. ova by ammonia is possible in fecal material stored in the solar toilet or any other dry toilet, if the following physical and chemical conditions of a closed vault with a minimum temperature of 28°C; an initial pH of 8.3, minimum moisture of 27.5%, and addition of 1% urea to the biosolid. At 28°C longer storage time would be required for 100% inactivation while at higher temperatures less time of storage would be necessary. [4] This current study is the field application of the work done in that study.

Other studies have evaluated the survival of pathogens in feces under storage and the association with the peak temperature and pH of the feces.[6, 27] Also, various others have studied the efficacy of using urea as an additive for sourcing ammonia for inactivation of pathogens in feces obtained from urine diverting toilets.[9] We have tried to study in this pilot project the use of urea as an additive to feces to source ammonia for inactivation of *Ascaris suum* eggs. The end point of the other studies has been 100% inactivation of the helminthic eggs and extended over a substantial period of time ranging from weeks to months. Based on the laboratory simulation work done by Cruz, we have tried to study the effects of shorter duration of storage in the real world scenario in generally uncontrolled conditions of temperature, duration of raised temperature and without a sustained preset temperature.[4] We did not achieve 100% inactivation of the *Ascaris suum* eggs as has previously been obtained in other studies. This is due the shorter treatment time (maximum 120 hours) compared to weeks or months in other studies. Also, the temperature was not controlled. Controlled temperature especially greater than 35^0 C has been shown to be rapidly effective in inactivation of fecal helminthic ova especially when coupled with ammonia.[4] A working strategy that uses urea as an ammonogen and reduces the storage time to a few days from the months and years required currently would enhance the safety profile of human biosolid and greatly decrease the risk of pathogen transmission due to flooding or mishandling of the stored feces. Also, the treated biosolid would be available for agricultural use much earlier. The current study has a few limitations. The first is the small sample size. Secondly the short treatment time was responsible for inadequate exposure to the intervention.

The fluctuant temperature that was not sustained at peak levels for prolonged periods of time led to less than optimal treatment as well. However, this is a good indicator of the real world scenario. Any future study would need to have longer duration of treatment to account for the irregularities of the temperature. We controlled for the pH and moisture at the beginning of the study, this could bias our findings since they may not be optimal in the real world. Future larger studies should consider these and possibly keep them uncontrolled. However, that would preclude demonstration of effectiveness of urea addition intervention due to dependence on pH and moisture for the formation of ammonia and inactivation of helminthic ova.

5.6 Conclusion

Soil transmitted helminthes are important neglected diseases that have a great morbidity in the poor developing countries. The search for an effective sustainable method for inactivation of helminthic ova is an ongoing one. Use of urea as an ammonogenic source for inactivation of helminthic ova is a promising strategy that is cheap and very effective in laboratory conditions. Our in-field study in real world scenario indicates the potential for the practical application of this strategy. However, large scale real world studies are further needed to evaluate this as an effective environmental control strategy for soil transmitted helminthes.

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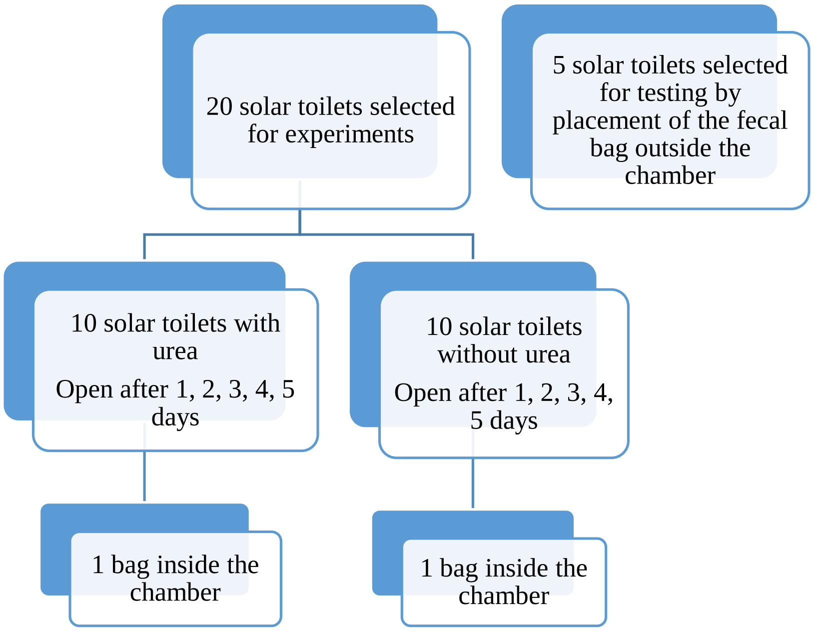

Figure 10. Experiment set-up for test of urea for inactivation of *Ascaris suum* eggs in solar toilets in El Salvador

Solar toilet inclusion criteria

1. Continuing use of toilet by household members
2. Adequate sun exposure; solar panel oriented to the south
3. Wall and floor of chamber should be of cement and brick
4. Solar panel adequately covering the chamber
5. Presence of urine diverting toilet seat
6. Urine diverting system working properly: no stagnant urine, no blockage of pipe
7. No presence of water/urine inside the chamber
8. Use of additive after each defecation

Box 1. Solar Toilet Inclusion Criteria

1. pH lower than 8
    a. Lime added
    b. Mixed fecal material
    c. Measured pH and repeated procedure until pH values are between 8.0 and 9.9.
2. pH higher than 10
    a. Soil added
    b. Mixed fecal material
    c. Measured pH and repeated procedure until pH values are between 8.0 and 9.9.
3. Moisture lower than 23%
    a. Water added
    b. Mixed fecal material
    c. Measured moisture and repeated procedure until moisture levels are between 23% and 50%.
4. Moisture higher than 50%
    a. Soil added
    b. Mixed fecal material
    c. Measured moisture and repeated procedure until moisture levels are between 23% and 50%.

Box 2. Schema for adjusting pH and moisture in feces.

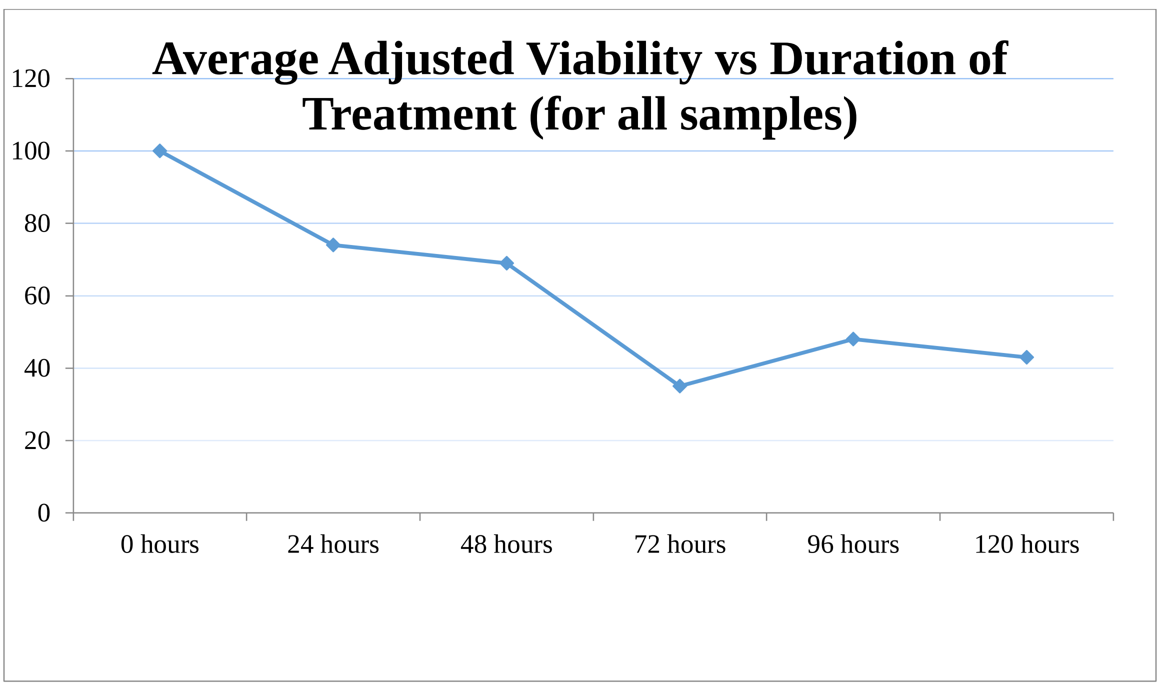

Figure 11. Overall Average adjusted viability of *Ascaris suum* eggs vs. Duration of Treatment.

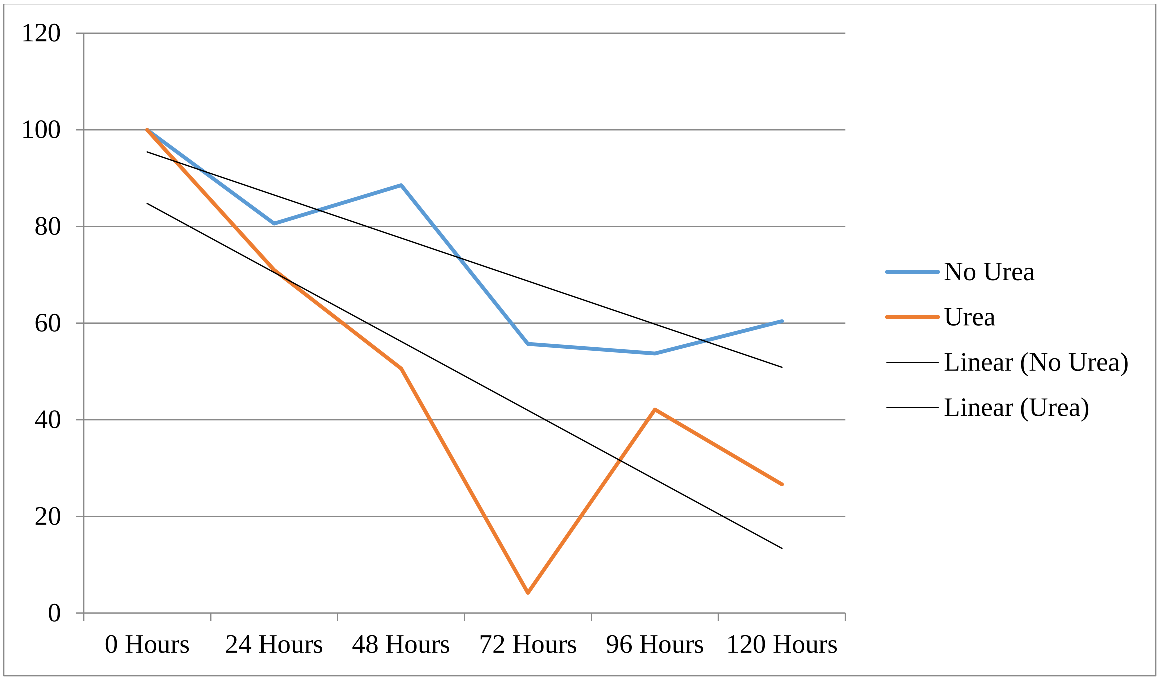

Figure 12. Comparison of Average adjusted viability of *Ascaris suum* eggs vs. Duration of Treatment with and without Urea Intervention.

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CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Based on the results of the studies, the following conclusions can be drawn for the aims listed in Chapter 1.

Aim 1: For each of the STH viz., *Ascaris lumbricoides*, *Trichuris trichiura* and Hookworm

- Aim1A: Determine prevalence in children aged 8-10 years in El Salvador

  Prevalence of *Ascaris lumbricoides*: 2.75%

  Prevalence of *Trichuris trichiura*: 4.1%

  Prevalence of Hookworm: 1.83%

- Aim1B: To determine association with personal, regional, water and sanitation, and behavioral risk factors

  1. For *Ascaris lumbricoides* infection

     - No association with personal factors (age and gender)

     - Regional factors (eco-epidemiological zone and rural-urban status)

       Association with Eco-epidemiologic zone. Volcanic chain and Central Depression were associated with higher risk of infections compared to Mountains.

       No Association with rural urban status

     - Water and Sanitation factors

Water source is significantly associated. ‘Spring or well’ water has higher risk of infection compared to piped water

Sanitation is significantly associated. ‘Open air’ defecation has higher risk of infection compared to ‘Septic tank or flush toilet’.

- Behavioral Factors

  Use of shoes significantly associated. Use of ‘sandals or nothing’ has higher risk of infection compared to ‘Always closed shoes’

2. For *Trichuris trichiura* infection

- No association with personal factors (age and gender)
- Regional factors (eco-epidemiological zone and rural-urban status)

  Association with Eco-epidemiologic zone. Coastal plains were associated with higher risk of infections compared to Mountains.

  Rural status was protective against infection

- Water and Sanitation factors

  Water source is significantly associated. ‘Spring or well’ water has higher risk of infection compared to piped water.

- Behavioral Factors

  Use of shoes significantly associated. Use of ‘sandals or nothing’ has higher risk of infection compared to ‘Always closed shoes’

3. For Hookworm infection

- No association with personal factors (age and gender)

- Regional factors (eco-epidemiological zone and rural-urban status)

  Rural status was protective against infection

- Water and Sanitation factors

  Water source is significantly associated. ‘Spring or well’ water has higher risk of infection compared to piped water.

- Behavioral Factors

  Poor handwashing was found to be protective.

  Use of shoes significantly associated. Use of ‘sometimes closed shoes’ has higher risk of infection compared to ‘Always closed shoes’

- Aim1C: To determine significant bio-behaviorally plausible interactions of risk factors

1. For *Ascaris lumbricoides*

   a. Multiplicative scale: Significant interactions identified between

      - Use of shoes and geophagy
      - Use of shoes and rural-urban status
      - Water and rural-urban status
      - Water and poor handwashing
      - Water and Sanitation

   b. Additive scale: Significant interactions identified between

      - Rural-urban status and Sanitation
      - Rural-urban status and Water

2. For *Trichuris trichiura*

   a. Multiplicative scale: Significant interactions identified between

      - Use of shoes and geophagy

- Use of shoes and poor handwashing
- Use of shoes and rural-urban status
- Rural-urban status and Sanitation
- Water and Sanitation
- Water and rural-urban status
- Water and poor handwashing

b. Additive scale: Significant interactions identified between

- Rural-urban status and Use of Shoes
- Rural-urban status and Sanitation
- Rural-urban status and Water

3. For Hookworm

a. Multiplicative scale: Significant interactions identified between

- Use of shoes and geophagy
- Use of shoes and poor handwashing
- Use of shoes and rural-urban status
- Rural-urban status and Sanitation
- Water and Sanitation
- Water and rural-urban status
- Water and poor handwashing

b. Additive scale: Significant interactions identified between

- Rural-urban status and Sanitation
- Rural-urban status and Water
- Water and Sanitation

- Aim1D: Determine association between supplier of piped water and infection

1. For *Ascaris lumbricoides*

- Significant association with type of piped water supplier
- Increased risk of infection with decentralized operators compared to ANDA
- Increased risk of infection with decentralized operators under contract management compared to ANDA
- Increased risk of infection with decentralized operators under contract management compared to decentralized operators not under contract management

2. For *Trichuris trichiura*
   - No association
3. For Hookworm
   - No association

Aim2: Determine inactivation of *Ascaris suum* ova by urea (1%) added to feces in solar toilets in field environment in the community

- Significant but weak association between the concentration of ammonia gas and inactivation rate of *Ascaris suum* ova is seen.
- Significant strong association with duration of treatment with urea is seen with the inactivation rate for *Ascaris suum* ova.
- Nearly 50% inactivation of *Ascaris suum* ova by urea 1% added to feces in solar toilets in field environment in the community.

6.2 Recommendations

1. The prevalence rate for each of the STH in El Salvador is less than 20%, so Mass Drug Administration program for STH to pre-school age children (pre-SAC) and school age children (SAC).

2. Eco-epidemiologic zones are very strongly associated with specific type of STH parasite. It suggests that control measures directed at a specific STH parasite should be focused on the eco-epidemiologic zone that has the most significant association with the STH parasite.

3. Rural residents were protected from infection by *Trichuris trichiura*. This suggests there is greater risk of infection in the urban regions. This could possibly be due to the lack of proper sanitation coverage and safe water access in the urban regions due to increased population density. It is therefore recommended that the improvement of the infrastructure in the urban regions should be carried out for control of *Trichuris trichiura* and hookworm. Given the specific mode of infection of hookworm by dermo-penetration, the urban regions have contamination of soil with infected feces. This coupled with higher population density is manifested with higher risk of hookworm infections.

4. Spring or well water has been associated with a high risk of infection with all three STH. This could be explained by the fact that these are surface water sources and probably unprotected, resulting in surface runoff contaminated with fecal matter. Also, the site for fetching water is usually not a built structure and the clayey soil becomes muddy when water splashes on it. This creates a moist environment for the infective forms of the STH to develop, the splashes of water can then contaminate the water in the container. Also, is the person fetching the water is not diligent, contamination through dirty hands and fingers can occur as well. The necessity of standing in the wet mud/ soil to fill water is an opportune time for infection by hookworm larvae. It is therefore recommended that the spring and well water sources be protected with embankments to avoid contamination with surface run-off. Also, concrete drains should be built around the perimeter to drain away the splashed water. It is also, very important to couple these measures with public health education measures. People need to be educated about the necessity for treating water collected from surface water sources.

5. Use of shoes is significantly associated with infection by all three STH parasites. For *Ascaris lumbricoides* and *Trichuris trichiura* the use 'sandals or nothing' compared to 'always closed footwear' is most significant while for hookworm it is the 'sometimes closed shoes' versus 'always closed footwear' that is most significant. It is evident that the best mode of protection from the STH parasite infection is through the use of 'always closed shoes'. It is therefore recommended that the public health education measures be implemented when formulating plan for the control of any of the three STH parasites that focusses on increasing the awareness of the people and children about the STH parasites and emphasizes the benefits of wearing 'always closed footwear'. These could be coupled with the education and training for children regarding handwashing with soap for maximal effect.

6. Based on the interaction testing, it is evident that for all of the three STH parasites - *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworms the interaction terms are significant and the risk factors that one must be aware of them in addition to the independent factors. For *Ascaris*, the important risk factors that have significant interactions are water source, type of sanitation, rural-urban status, and use of shoes, poor handwashing, and geophagy. Interventions directed at control of *Ascaris lumbricoides* ought to be with cognizance of these significant interactions to ensure most optimal resource allocation for the best results.

7. Based on the significant parameter estimate for the duration of treatment with urea for the inactivation of *Ascaris suum* ova, it is evident that the longer duration of treatment is associated with a greater inactivation of *Ascaris suum* ova. Urea has been shown to be very effective in inactivation of *Ascaris suum* ova in laboratory conditions. Our study shows its potential for use in the field under the uncontrolled conditions of temperature with significant inactivation achieved in a very short storage time. It is there recommended that further studies to determine the efficacy of urea as a field agent for inactivation of fecal pathogens be carried out. It is cheap, easily available and socially acceptable.

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