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Inactivation of Ascaris in Double-Vault Urine-Diverting Composting Latrines in Panama: Methods and Environmental Health Engineering Field Applications

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Inactivation of *Ascaris* in Double-Vault Urine-Diverting Composting Latrines in Panama:

Methods and Environmental Health Engineering Field Applications

by

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ABSTRACT

The United Nations Millennium Development Goals have prioritized improving access to sanitation, but unfortunately about a third of the global population is still without an improved sanitation source and one billion still practice open defecation. Lack of access to adequate and safe sanitation means the proliferation of dangerous pathogens in the environment, especially soil-transmitted helminths (STHs). In the Bocas del Toro Province of Panama (and similar locations in the world), composting latrines have been built in many of the indigenous communities in the area. They are a form of dry or ecological sanitation and are designed to produce an end product that can be used as a soil amendment for agricultural purposes. The issue is that many of these latrines are not working as designed and do not go through the composting process. Instead, they may act as incubators for harmful pathogens, such as *Ascaris lumbricoides* (roundworm).

This research 1) provides an extensive literature review of the health situation of Panama, focusing on indigenous populations; soil-transmitted helminths and helminthiasis; *Ascaris lumbricoides* and its implications for wastewater reuse and land application of biosolids/sewage sludge; and inactivation of *Ascaris* in composting latrines; and 2) develops and proposes an experimental plan, with field-based methods, to assess the inactivation of *Ascaris,* by urea and solar heat (increased temperature), in composting latrines in Panama. Various experiments have been conducted in the laboratory using urea and increased temperature to inactive *Ascaris*; however few have been carried out in dry toilet technologies in the field. The contribution of this thesis is the field-based experimental design developed for

inactivating *Ascaris* in composting latrines. The methods build upon previous research carried

out both in the laboratory and in the field.

CHAPTER 1: INTRODUCTION, MOTIVATION, OBJECTIVES

1.1 Introduction

As the 2015 target date for the United Nations Millennium Development Goals (MDGs) draws near, the lack of access to safe sanitation is still a major global public health problem with significant regional and urban/rural disparities. According to the latest World Health Organization (WHO) / United Nations Children's Fund (UNICEF) Joint Monitoring Programme for Water Supply and Sanitation Update Report (2013), 2.5 billion people were still without access to any form of improved sanitation technology or facility in 2011. Improved sanitation is defined as facilities that "are likely to ensure hygienic separation of human excreta from human contact" (World Health Organization [WHO] & UNICEF, 2013). The JMP Update Report points out that, throughout the world, over a billion people (15% of the global population) still use open defecation as their form of sanitation (WHO &UNICEF, 2013). The specific MDG that pertains to provision of sanitation is MDG 7, which has to do with ensuring environmental sustainability. One of its targets (Target 7c) is to "halve, by 2015, the proportion of the population without sustainable access to safe drinking water and basic sanitation" (United Nations [UN], n.d.). However, in order to meet the sanitation portion of this target, the global population would need to provide 1 billion more people with an improved sanitation source by 2015 (WHO & UNICEF, 2013).

Table 1 provides information on global rates of sanitation coverage by region, while demonstrating regional-based disparities. Table 2 contains data on global sanitation coverage in rural and urban areas and demonstrates disparities between these two populations. In order

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to measure progress towards Target 7c, and for purposes of comparability of data, sanitation type is broken down into four different categories. These categories make up what is referred to as the sanitation ladder, and are based on whether the facility is considered to be an improved or unimproved sanitation source. The bottom rung of the ladder contains improved sanitation facilities, which include: flush/pour flush toilets to piped sewer systems, septic tanks, and pit latrines; ventilated improved pit latrines (VIP); pit latrines with a slab; and composting toilets. The next three rungs on the sanitation ladder include forms of unimproved sanitation. The first of these are shared sanitation facilities, which are "sanitation facilities of an otherwise acceptable type shared between two or more households. Only facilities that are not shared or not public are considered improved" (WHO &UNICEF, 2013). The next rung contains unimproved facilities that "do not ensure hygienic separation of human excreta from human contact. Unimproved facilities include pit latrines without a slab or platform, hanging latrines, and bucket latrines" (WHO & UNICEF, 2013). At the top of the sanitation ladder is open defecation, which is the process of human feces being disposed of in "fields, forests, bushes, open bodies of water, beaches or other open spaces or disposed of with solid waste" (WHO &UNICEF, 2013).

Open defecation and other forms of unimproved sanitation are of public health concern because of the pathogens that can be found in contaminated human feces. These pathogens include: viruses, bacteria, protozoa, and helminthes (Feachem, Bradley, Garelick, & Mara, 1983). Feces need to be contained and/or treated through improved sanitation technologies in order to ensure sufficient pathogen destruction and reduction in human exposure to these pathogens (CDC, 2012). Soil-transmitted helminths (STHs) are of particular public health concern, and include: *Ascaris lumbricoides* (roundworm), *Trichuris trichiura* (whipworm), and the two forms of human hookworm, *Ancylostoma duodenale* and *Necator americanus* (Pan American Health Organization [PAHO], 2014).

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Table 1. Proportion of Sanitation Coverage by Region, 2012 (data from WHO & UNICEF, 2014)

	Southern Asia	Sub-Saharan Africa	South-Eastern Asia	Oceania	Northern Africa	Latin America and the Caribbean	Western Asia	Eastern Asia	Caucasus and Central Asia	World
Open Defecation	38%	25%	13%	12%	3%	3%	3%	1%	0%	14%
Other Unimproved	9%	26%	6%	48%	0%	8%	4%	13%	2%	11%
Shared	11%	19%	10%	5%	6%	7%	4%	19%	3%	11%
Improved	42%	30%	71%	35%	91%	82%	89%	67%	95%	64%

Table 2. Proportion of Global Urban and Rural Sanitation Coverage, 2012 (data from WHO & UNICEF, 2014)

	Open Defecation	Other Unimproved	Shared	Improved
Urban	3%	4%	13%	80%
Rural	27%	17%	9%	47%

In many parts of the world, the most persistent of pathogens found in human waste is *A. lumbricoides* (WHO, 1989). It is currently estimated that approximately 807-1,121 million people are infected with roundworm (*Ascaris*), as compared to 604-795 million infected with whipworm, and 576-740 million infected with hookworm (CDC, 2013b), and it is predicted that globally between four billion (CDC, 2011) and 5.3 billion people are at risk for infection with one or more of these STHs (Pullan & Brooker, 2012). Soil-transmitted helminthiasis is a part of a group of diseases known as Neglected Tropical Diseases (NTDs), and NTDs are typically found amongst the most impoverished people in the world living in tropical and subtropical regions. NTDs have traditionally been ignored by the development world and are significant because they can lead

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to severe morbidity and mortality. Fortunately, they can be controlled or eliminated, and in the case of STHs, safe water, proper sanitation and other public health interventions, such as deworming, are key in reducing the global prevalence of disease (WHO, 2010). Helminthiasis is thus an infection of the poor, infecting people in tropical and subtropical regions where sanitation and hygiene are inadequate (PAHO, 2014). Control of STHs is crucial in meeting the MDGs, and seven of the eight goals would be impacted if mothers and children were free of these worms (Inter-Development Bank [IDB], PAHO, & Sabine Vaccine Institute, 2011). In specific, one control strategy alone, that of deworming (also known as chemotherapy prophylaxis), when carried out on a regular basis, can help achieve these goals (WHO, 2005).

Many areas around the world have historically used and/or currently use untreated or partially treated wastewater as a source of water and nutrients for food production, which can pose a major risk to public health (Verbyla, Oakley, & Mihelcic, 2013). Global stressors such as climate change, population increase, water scarcity, food insecurity, and unsustainable practices have created a major need for the recovery of resources globally. There is a need to develop sustainable, effective, and safe sanitation solutions where resources can be recovered from biosolids and wastewater for agricultural purposes, without causing reinfection of the environment or humans with harmful and persistent pathogens, such as *A. lumbricoides*. The provision of sanitation must therefore be done in a manner that allows for both resource recovery and the protection of the public's health. One such example currently employed in many areas of the world is the use of dry sanitation technologies, such as urine-diverting composting latrines (Mihelcic, Myre, Fry, Phillips, & Barkdoll, 2009). These dry toilets do not use water and are a type of ecological or eco-sanitation solution in which the contents can be used as fertilizer or soil amendment.

This thesis focuses on Double-Vault Urine-Diverting (DVUD) composting latrines because of their use as an improved sanitation technology in coastal areas of Central America

where the author of this thesis served as a Peace Corps Volunteer as part of the Master's International Program (Mihelcic, Phillips, & Watkins, 2006). In addition, previous research has demonstrated these types of latrines to be ineffective in inactivating STH ova, particularly *Ascaris* eggs, (Corrales, Izurieta, & Moe, 2006; Mehl, Kaiser, Hurtado, Gibson, Izurieta, & Mihelcic, 2011) because they are not easily inactivated and are very persistent in the environment (Feachem et al., 1983). Due to the severe morbidity helminthiasis can cause, the high prevalence of infection in humans, and the widespread global distribution of this etiological agent, it is necessary to find effective and sustainable sanitation solutions that inactivate STH eggs in order to protect both the environment and the public's health.

1.2 Motivation and Objectives

1.2.1 Motivation

The motivation for this thesis is based on the need to provide increased sanitation coverage, in an environmentally sustainable manner, to the 2.5 billion people who still do not have access to an improved sanitation source, as well as reducing helminthiasis morbidity and mortality caused by unsafe and inadequate water and sanitation sources and hygiene practices. The idea is to accomplish this through the use of eco or dry sanitation technologies (such as composting latrines) in geographic locations within less-developed countries that do not allow for other types of sanitation to be used (i.e. flush toilet, pour flush toilet, pit latrine, etc.) due to high water tables, flooding issues, water scarcity, and/or limitations in economic resources. Wastewater and excreta contain valuable resources such as carbon, nitrogen, and phosphorus, and these resources can be recovered in a composting latrine. Therefore, if safe use of waste residuals can be ensured, the composted solids can be used in agriculture as a soil amendment in tropical regions where farming is the livelihood of many. By making health and resource recovery compatible, there should be a positive impact on reducing parasitic infection, global food insecurity, water scarcity, and malnutrition.

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1.2.2 Objectives

The objectives for this research are to: 1) provide an extensive literature review of the health situation of Panama, focusing on indigenous populations; soil-transmitted helminths and helminthiasis; *Ascaris lumbricoides* and its implications for wastewater reuse and land application of biosolids/sewage sludge; and inactivation of *Ascaris* in composting latrines; and 2) develop and propose an experimental plan, with field-based methods, to assess the inactivation of *Ascaris,* by urea and solar heat (increased temperature), in composting latrines in Panama. Numerous experiments have been conducted in the laboratory using urea and increased temperature to inactive *Ascaris*; however very few have been carried out in dry toilet technologies in the field. The contribution of this thesis is the field-based experimental design developed for inactivating *Ascaris* in composting latrines. The methods build upon previous research carried out both in the laboratory and in the field.

Chapter 2 provides an overview of the health situation in Panama, detail on the lifecycle and epidemiology of STH infection and related health effects, risk factors, and control strategies. In addition, the chapter examines *Ascaris* in wastewater and sewage sludge a well as studies carried out related to the inactivation of *Ascaris* eggs both in the laboratory and in dry toilet technologies in the field. *Ascaris* inactivation by storage and temperature, alkaline stabilization and dehydration, and urea will be examined. Chapter 3 provides detailed methods for a field-based experiment that can be carried out in coastal indigenous communities in Panama where a large quantity of composting latrines have been built, but very few have been tested for their ability to inactivate STHs. Finally, Chapter 4 will provide recommendations for environmental public health engineers carrying out composting latrine projects in the field in Latin America and the Caribbean.

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CHAPTER 2: LITERATURE REVIEW

2.1 Background on Panama

2.1.1 Brief Geographic, Socio-Economic, and Demographic Situation of Panama

The Republic of Panama is a country in Latin America bordered by Costa Rica on the West, Colombia on the East, the Caribbean Sea on the North, and the Pacific Ocean on the South. The Panama Canal links both bodies of water by bisecting the mainland into eastern and western halves (PAHO, 2012). Figure 1 demonstrates that Panama is made up of nine provinces and three main indigenous provincial territories known as Comarcas (Comarcas Ngäbe-Buglé, Emberá-Wounaan, and Kuna Yala).
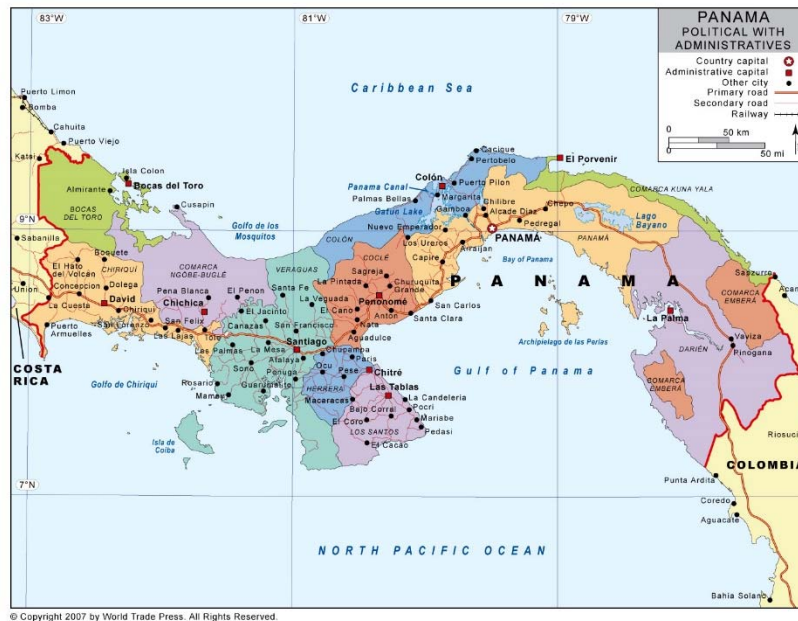
Figure 1. Map of the Republic of Panama with Provinces and Comarcas (Maps Courtesy of World Trade Press: Copyright © 2013 World Trade Press. All Rights Reserved. Royalty Free Standard License)

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In the interior of the country, the terrain is mostly mountainous with some upland plains, and the coastal areas are made up of plains and rolling hills (Central Intelligence Agency (CIA), 2014). Seventy percent of the land has an elevation of 700 meters or less, and the extensive Pacific and Caribbean coastlines include 1,518 islands (Autoridad Nacional del Ambiente-Panamá [ANAM], Contraloría General de la República Instituto Nacional de Estadística y Censo [INEC], & Programa de las Naciones Unidas para el Medio Ambiente [PNUMA], n.d.). Panama's climate is tropical maritime, which means it is hot, humid, and cloudy, with a long rainy season and short dry season each year (CIA, 2014).

The total estimated population of Panama for 2013 was 3,864,200 people, and the percentage of the total population living in urban areas was 76.4% (PAHO, n.d.), concentrated mostly in the Colón and Panamá Provinces. These two provinces are part of the Interoceanic Region (Canal Zone), and provide greater job opportunities and access to services (Ministerio de Salud Panamá, 2013). Indigenous groups make up approximately 12% of the overall population (2010 estimates) (PAHO, 2012), and the three largest indigenous groups are the Ngäbe-Buglé, Emberá-Wounaan, and Kuna Yala, each with their own Comarca. The Provinces of Bocas del Toro and the Darién also have a significant indigenous population (Ministerio de Salud Panamá, 2013). The population breakdown by Province and Comarca is noted in Table 3.

Panama is classified as an upper-middle-income country (PAHO, 2012) with a fast growing economy. It is in the process of undergoing a demographic transition, and over the past few years, the overall poverty rate has declined by about 10%. Despite this economic prosperity, major poverty and inequalities still exist. Panama has the second worst income distribution in Latin America (CIA, 2014), and in 2012, 25.8% of the total population was impoverished, and 10.4% suffered from extreme poverty. Inequalities exist between rural and urban populations, as well as within the Comarcas and amongst other Provinces that have large

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Table 3. Population by Province and Comarca, 2012 (INEC, n.d.)

Province	Population
Bocas del Toro	143,232
Coclé	249,823
Colón	263,659
Chiriquí	442,058
Darién	52,368
Herrera	117,193
Los Santos	94,562
Panamá	1,949,063
Veraguas	241,555
Comarca Kuna-Yala	39,950
Comarca Emberá-Wounaan	11,125
Comarca Nägbe-Buglé	182,923

numbers of indigenous people, such as the Darién and Bocas del Toro. The 2012 rural poverty

rate for the entire country was 50.2% compared to 12.6% for urban areas, and the extreme

poverty rate for rural areas was 24.3% and for urban areas was 2.9%. The largest inequality

exists for those living in the three Comarcas, as 89.8% of the population were poor in 2012 and

68.5% were in extreme poverty. Significant health disparities include the infant death rate,

children under five death rate, and maternal mortality. Table 4 demonstrates inequalities

Table 4. Infant, Children under 5, and Maternal Mortality Rates per 1,000 Live Births, 2011
(Adapted from Ministerio de Salud Panamá, 2013)

	Panamá (Country)	Bocas del Toro	Darién	Comarca Kuna Yala	Comarca Ngäbe-Buglé
Infant Mortality Rate (per 1,000 live births)	13.2	20.1	19.1	19.5	20.8
Children Under 5 Mortality Rate (per 1,000 live births)	16.66	30.26	23.66	30.37	32.65
Maternal Mortality Rate (per 1,000 live births)	80.5	158.0	76.3	542.3	300.5

suffered by the Comarcas, and Darién and Bocas del Toro Provinces, as compared to the Country-level rates (Ministerio de Salud Panamá, 2013).

2.1.2 Water and Sanitation Coverage and Enteric Disease

Access to improved water and sanitation sources are imperative in reducing diarrheal disease, helminthasis, and malnutrition. The latest (2012) coverage estimates for Panama at the country level according to WHO & UNICEF (2014) are listed in Tables 5 and 6. These estimates mirror the disparities seen at a global level; that sanitation coverage, as a whole, lags behind water coverage and that major disparities exist between urban and rural populations. In addition, Table 7 provides 2010 data for the provinces and comarcas in Panama with the least amount of water and sanitation coverage.

Diarrheal disease and intestinal parasitic infection are major causes of morbidity in Panama, where as reported mortality due to diarrheal disease occurs at a lesser extent. The mortality rate for children under 5 due to acute diarrheal disease was 0.18 per 1,000 inhabitants, with 66 reported deaths occurring in 2012. The second most common cause of morbidity in Panama is due to diarrhea and gastroenteritis, with a rate of 3,063.4 per 100,000 inhabitants, and 116,029 reported cases occurring in 2012. Intestinal parasitosis was the ninth most

Table 5. Sanitation Use by Source Type, Panama, 2012 (Adapted from WHO & UNICEF, 2014)

	Sanitation Type	Urban	Rural	Country
Improved	*Improved*	80%	52%	73%
Unimproved	*Shared*	9%	5%	8%
	Other Unimproved	10%	30%	15%
	Open Defecation	1%	13%	4%

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common cause of morbidity, with 49,188 reported cases and a rate of 1298.7 per 100,000 inhabitants, and non-specified gastritis was the thirteenth most common with 32,859 reported cases and a rate of 867.6 per 100,000 inhabitants (Ministerio de Salud Panamá, 2014).

Table 6. Water Use by Source Type, Panama, 2012 (Adapted from WHO & UNICEF, 2014)

	Water Type	Urban	Rural	Country
Improved	*Total Improved*	97%	86%	94%
Improved	*Piped Water on Premise*	95%	83%	92%
Improved	*Other Improved*	2%	3%	2%
Unimproved	*Other Unimproved*	3%	4%	4%
Unimproved	*Surface Water*	0%	10%	2%

Table 7. Percentage of Population with Improved Water and Sanitation Sources for Provinces and Comarcas with Lowest Coverage, 2010 (data from Ministerio de Salud Panamá, 2013)

	Bocas del Toro	Darién	Comarca Kuna Yala	Comarca Ngäbe-Buglé	Comarca Emberá-Wounaan
Improved Sanitation Source	82%	75%	6%	41%	58%
Improved Water Source	76%	71%	77%	41%	28%

2.2 Background on Soil-Transmitted Helminths

2.2.1 Transmission and Lifecycle

STHs are parasitic nematode worms that cause infection in humans (Bethony et al., 2006) in tropical and subtropical regions. They include: *Ascaris lumbricoides* (roundworm), *Trichuris trichiura* (whipworm), and the two forms of human hookworm, *Ancylostoma duodenale* and *Necator americanus* (PAHO, 2014). Unembryonated eggs are found in the feces of infected individuals and are deposited into soil where sanitation is inadequate (WHO, 2014). STH infection occurs when people ingest soil contaminated with embryonated roundworm and whipworm eggs in their infective stage. Hookworm eggs are not infective, but their larvae are. The main route of transmission for both types of human hookworm is through the skin, which primarily occurs when walking barefoot in contaminated areas. One type of hookworm, *A. duodenale*, is also transmitted by ingesting the infective larvae (CDC, 2013b).

Direct person-to person transmission of STH infections does not occur because fresh feces do not contain infective STH eggs (WHO, 2014). The eggs require a cycle in the soil in order to become infective.   Ingestion of STHs in their infective stages occurs via numerous routes that include: placing hands that contain contaminated soil in the mouth; via the consumption of contaminated food (i.e. fruits and vegetables) that have not been adequately peeled, washed, or cooked (CDC 2013b); and by contaminated water sources (PAHO, 2003). The risk of transmission due to contaminated food may be increasing globally with the reuse of wastewater for irrigation of crops and as an organic fertilizer. Furthermore, the use of human biosolids as fertilizer has demonstrated an elevated output of *Ascaris* eggs in feces (Scott, 2008).

Each STH has a different lifecycle, although all primarily parasitize the intestines, either in the small or large intestines depending on the parasite. *A. lumbricoides* is found throughout the small intestine, whereas *T. trichiura* is found in the large intestine (most often in the

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caecum), and hookworm parasitizes the upper part of the small intestine (Bethony et al., 2006).
After ingesting viable *Ascaris* eggs, the protective outer layer dissolves in the stomach as a
result of contact with gastric acid. The larvae are released into the first part of the small
intestine, the duodenum, and then travel to the caecum (the first section of the large intestine),
where they penetrate the intestinal mucosa. The larvae then travel to the liver and on to the
lungs. They travel up towards the epiglottis and are coughed up and swallowed. They reenter
the gastrointestinal tract and develop into adult worms in the small intestine. Fertilized female
worms begin laying eggs between nine and eleven weeks after egg ingestion (Bethony et al.,
2006; Guerrant, Walker, & Weller, 2011a). After the ingestion of *Trichuris* eggs, the larvae are
released in the stomach and make their way into the caecum of the large intestine. The larvae
penetrate the epithelium and molt, developing into adult whipworms approximately twelve
weeks after infection (Bethony et al., 2006; Guerrant et al., 2011c). *Necator* larvae (hookworm)
hatch from eggs found in soil and molt twice to become infective. The infective larvae enter the
human host through the skin and reach the lungs via the venules and lymphatic system. They
pass over the epiglottis and enter into the gastrointestinal tract where they enter the small
intestine, molt again, and develop into adults. The female worms begin releasing eggs five to
nine weeks after the larvae penetrate the skin. *Ancylostoma* larvae (hookworm) enter the host
through the skin and also through ingestion. In addition, the larvae may enter mammary glands
and be transmitted during breastfeeding (Bethony et al., 2006; Guerrant et al., 2011b). An
overview of the biology and transmission of each STH is noted in Table 8. Figures 2 through 4
contain Lifecycle diagrams for *A. lumbricoides* (roundworm), *T. trichiura* (whipworm), and the
two forms of human hookworm, *A. duodenale* and *N. americanus*, respectively.

Table 8. Overview of Roundworm, Whipworm, and Hookworm Biology and Transmission

Parasite	Time for eggs to become infective in soil	Lifespan in human host	Length of mature worm	Fecundity (daily egg output per female worm)	Location in host	Mode of transmission
Ascaris lumbricoides	2-4 weeks in warm, moist, shady soil [4]	1 year [1] 1-2 years[7]	15-40cm[1] 20-49cm[4] Male: 15-20cm[6] Female: 20-35 cm[6]	200,000[1] 240,000[4] Over 200,000[6]	Entire Small Intestine[1] Jejunum[6]	Ingestion of embryonated eggs[2] [4]
Trichuris trichiura	2-4 weeks[3]	1.5 - 2 years[1] 1-3 years[3]	3-5 cm[1]	3,000-5,000[1] 3,000-20,000[3]	Caecum and Colon (Large intestine)[1]	Ingestion of embryonated eggs[2] [3]
Necator americanus	7-10 days[5]	5-7 years[1] 3-5 years[5]	0.7-1.3 cm[1] Male: 0.7-0.9cm[5] Female: 0.9-1.1cm[5]	9,000-10,000[1] 5,000-10,000[5]	Upper Small Intestine[1]	Skin penetration with larvae[2] [5]
Ancylostoma duodenale	7-10 days[5]	5-7 years[1] 1 year[5]	0.8-1.3 cm[1] Male: 0.8-1.1cm[5] Female: 1.0-1.3 cm[5]	25,000-30,000[1] 10,000-30,000[5]	Upper Small Intestine[1]	Skin penetration and ingestion of larvae[2] [5]

Data from: Bethony et al., 2006 (1); CDC, 2013b (2); Guerrant et al., 2011c (3); Guerrant et al., 2011a (4); Guerrant et al., 2011b (5); O'Lorcain & Holland, 2000 (6); Scott, 2008 (7)

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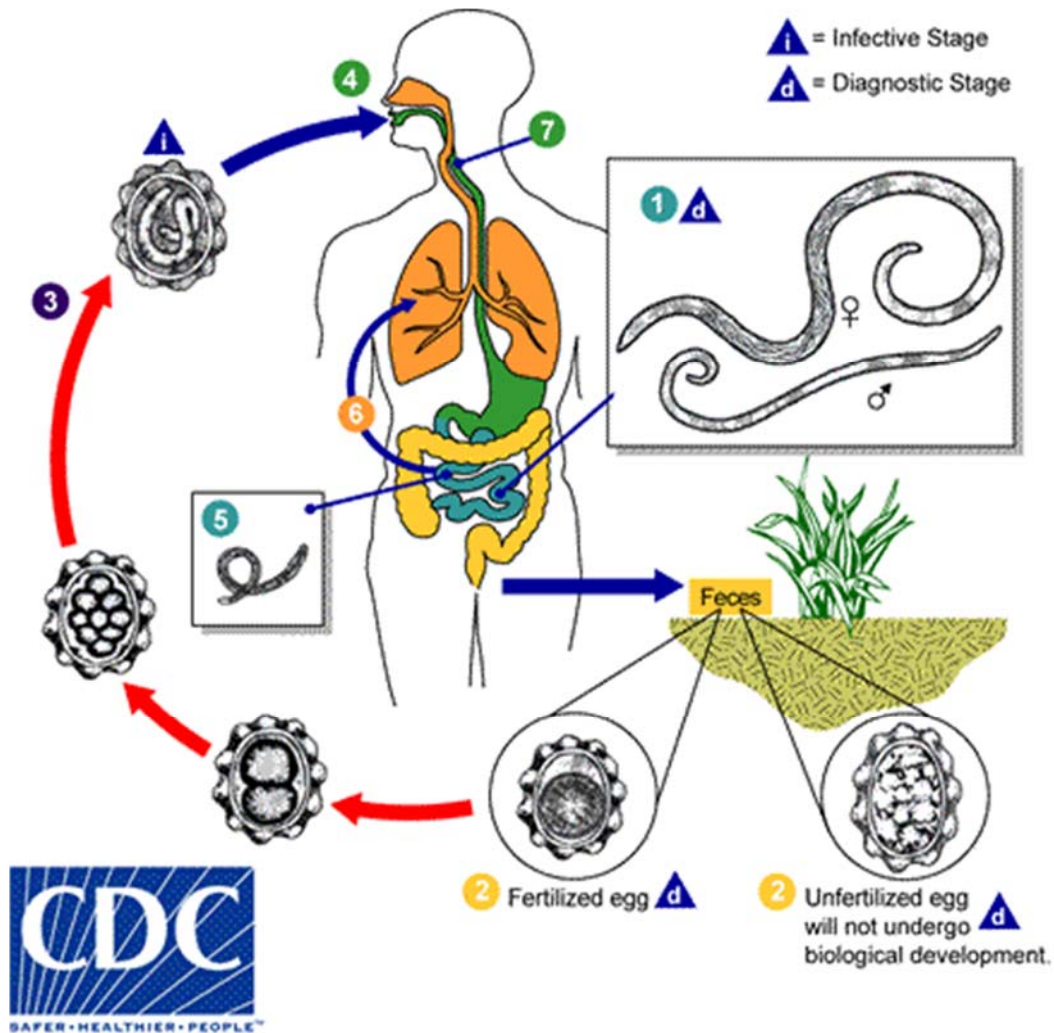
Adult worms ❶. live in the lumen of the small intestine. A female may produce approximately 200,000 eggs per day, which are passed with the feces ❷. Unfertilized eggs may be ingested but are not infective. Fertile eggs embryonate and become infective after 18 days to several weeks ❸, depending on the environmental conditions (optimum: moist, warm, shaded soil). After infective eggs are swallowed ❹, the larvae hatch ❺, invade the intestinal mucosa, and are carried via the portal, then systemic circulation to the lungs ❻. The larvae mature further in the lungs (10 to 14 days), penetrate the alveolar walls, ascend the bronchial tree to the throat, and are swallowed ❼. Upon reaching the small intestine, they develop into adult worms. Between 2 and 3 months are required from ingestion of the infective eggs to oviposition by the adult female. Adult worms can live 1 to 2 years.

Figure 2. Lifecycle of *A. lumbricoides* (Roundworm) (Retrieved from CDC, 2013a)
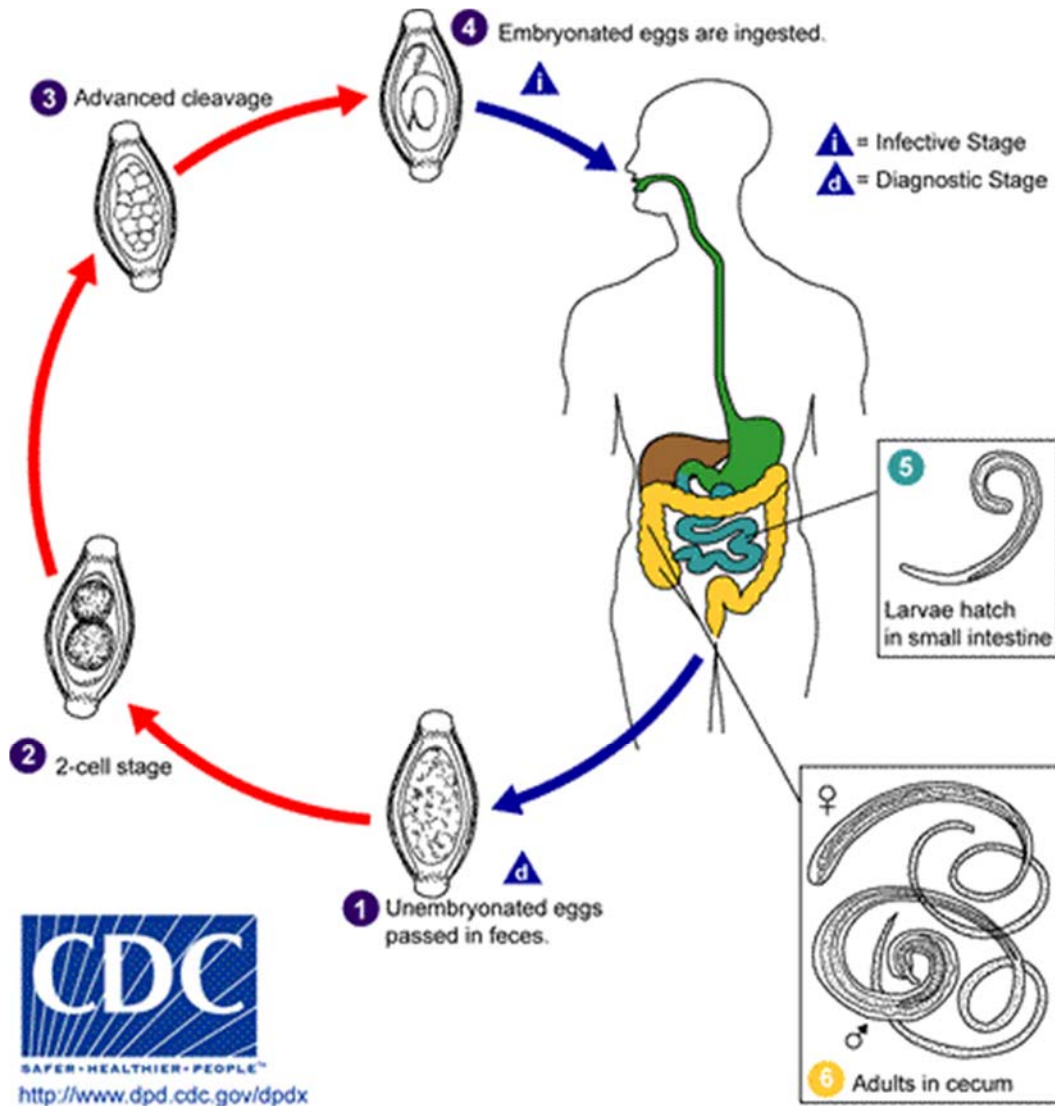
The unembryonated eggs are passed with the stool ❶. In the soil, the eggs develop into a 2-cell stage ❷, an advanced cleavage stage ❸, and then they embryonate ❹; eggs become infective in 15 to 30 days. After ingestion (soil-contaminated hands or food), the eggs hatch in the small intestine, and release larvae ❺ that mature and establish themselves as adults in the colon ❻. The adult worms (approximately 4 cm in length) live in the cecum and ascending colon. The adult worms are fixed in that location, with the anterior portions threaded into the mucosa. The females begin to oviposit 60 to 70 days after infection. Female worms in the cecum shed between 3,000 and 20,000 eggs per day. The life span of the adults is about 1 year.

Figure 3. Lifecycle of *T. trichiura* (Whipworm) (Retrieved from CDC, 2013a)
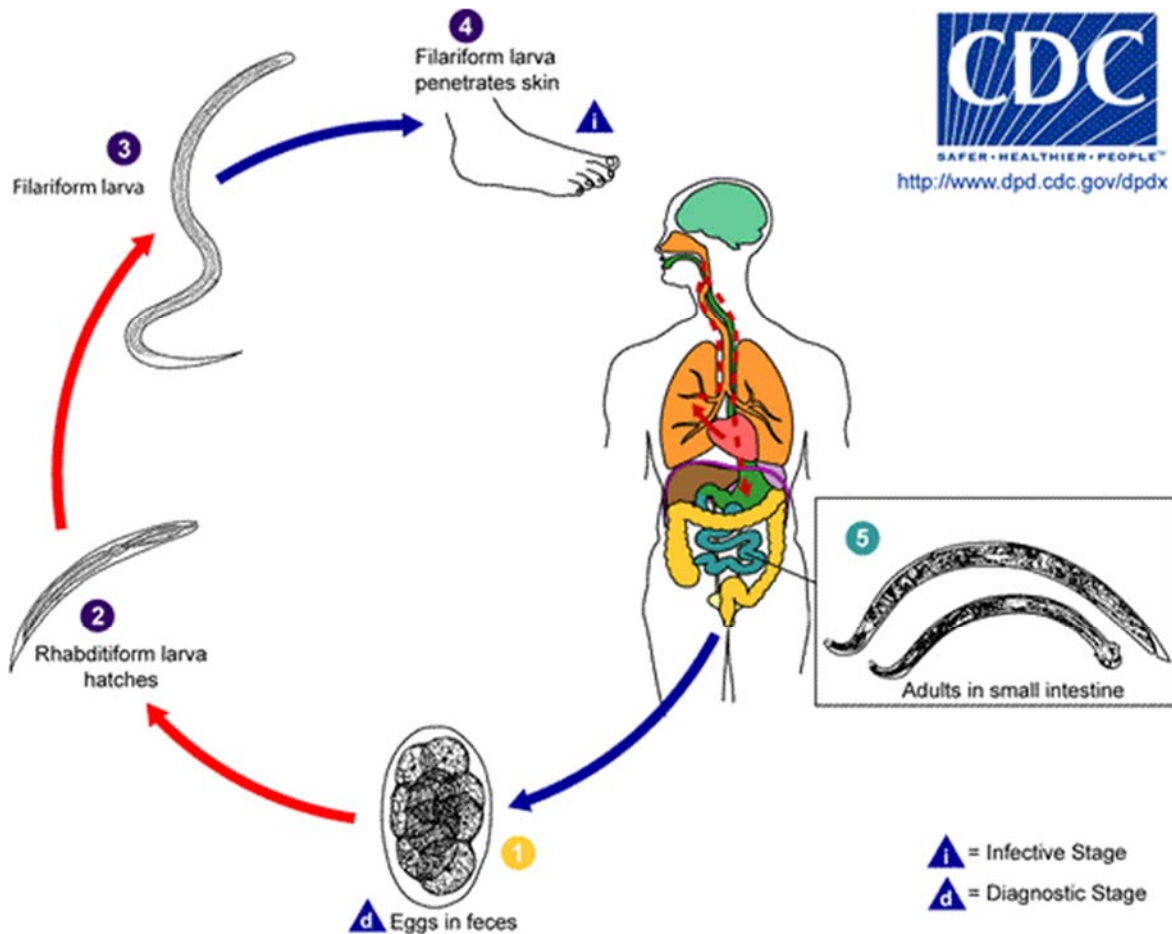
Eggs are passed in the stool ❶, and under favorable conditions (moisture, warmth, shade), larvae hatch in 1 to 2 days. The released rhabditiform larvae grow in the feces and/or the soil ❷, and after 5 to 10 days (and two molts) they become filariform (third-stage) larvae that are infective ❸. These infective larvae can survive 3 to 4 weeks in favorable environmental conditions. On contact with the human host, the larvae penetrate the skin and are carried through the blood vessels to the heart and then to the lungs. They penetrate into the pulmonary alveoli, ascend the bronchial tree to the pharynx, and are swallowed ❹. The larvae reach the small intestine, where they reside and mature into adults. Adult worms live in the lumen of the small intestine, where they attach to the intestinal wall with resultant blood loss by the host ❺. Most adult worms are eliminated in 1 to 2 years, but the longevity may reach several years. Some *A. duodenale* larvae, following penetration of the host skin, can become dormant (in the intestine or muscle). In addition, infection by *A. duodenale* may probably also occur by the oral and transmammary route. *N. americanus*, however, requires a transpulmonary migration phase.

Figure 4. Lifecycle of *A. duodenale* and *N. americanus* (Hookworm) (Retrieved from CDC, 2013a)

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2.2.2 Prevalence and Related Health Effects

Soil-Transmitted Helminthiasis is the most common infection of the poor in Latin America and the Caribbean (LAC), and in some marginalized areas, prevalence rates can be as high as 90% of the population infected. The impoverished and extremely impoverished that live in rural areas, and in shanty towns or slums in cities and periurban areas, are disproportionately at risk for STH infection, especially indigenous and Afro-descendent populations and communities (IDB et al., 2011). Globally, the CDC (2013b) estimates that approximately 807-1,121 million people are infected with roundworm, 604-795 million with whipworm, and 576-740 million with hookworm. Between four billion (CDC, 2011) and 5.3 billion people are believed to be at risk for infection with at least one of these STHs (Pullan & Brooker, 2012).

In LAC, it is estimated that in 2010, 86 million were infected with *A. lumbricoides*, 72.2 million with *T. trichiura*, and 30.3 million infected with hookworm. The Central Latin American sub-region, which includes Panama, is estimated to have to the largest number of people infected with STHs in the LAC region. It is estimated that in 2010, 41.8 million were infected with *A. lumbricoides*, 44 million with *T. trichiura*, and 13.5 million infected with hookworm (Pullan, Smith, Jasrasaria, & Brooker, 2014). In 2009, the WHO (2012) considered Panama to be a country with a moderate burden of soil-transmitted helminthiasis. This means that the proportion of Preschool-Aged Children (PSAC), aged one through four years old, and School-Aged Children (SAC), aged five through fourteen years old, requiring preventive chemotherapy (deworming medication) was between one-third and two-thirds of the population. Children are most at risk, and 124,321 Panamanian PSAC and 299,996 SAC were in need of preventive chemotherapy according to 2009 estimates. Despite these numbers, Panama does not have a mass drug administration (MDA) program in place (WHO, 2012).

Soil-Transmitted Helminthiasis is a great concern because of the morbidity associated with the infections. Those with light infections usually do not have any symptoms. The severity

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of the morbidity is related to the number of worms harbored in an individual, and people with heavier infections suffer a range of symptoms (WHO, 2014). It is estimated that 20% of the population contributes to 80% of the STH burden in an area (IDB et al., 2011). Since morbidity and rate of transmission are directly related to worm burden, the intensity of infection is the main epidemiological index used to describe infection with STHs (Bethony et al., 2006). The primary form of morbidity due to STH infection is nutritional impairment (WHO, 2010), including malnutrition (Bethony et al., 2006), and this impairment impacts the growth and physical development of children (WHO, 2014). STH infection causes cognitive and intellectual impairment, educational deficits (Bethony et al., 2006), and necessitates possible surgical intervention if complications arise (WHO, 2010). Soil-transmitted helminthiasis directly affects school performance, attendance, and future economic productivity. In addition, STH infections might increase the susceptibility of the host to, or exacerbate the risk of infection with, other diseases such as HIV infection, malaria, and tuberculosis (Bethony et al., 2006; Weaver, Hawdon, & Hoberg, 2010). Pregnant women and their babies are susceptible to serious negative health effects caused by STHs, including life-threatening anemia. Intestinal worms rob nutrients from the mothers, which hinders growth of the fetuses and causes low birth weight in the newborn infants (IDB et al., 2011).

Individuals with light infections of *A. lumbricoides* may not show any symptoms besides abdominal discomfort (CDC, 2013b). However, moderate to heavy infections can produce numerous negative health effects. Migration of the larvae to the lungs causes: acute lung inflammation (pneumonitis), difficulty in breathing, wheezing, dyspnea, nonproductive cough, sputum containing blood, high fever, urticarial rash, and angioedema. In addition, infection with *A. lumbricoides* results in: abdominal distension and pain, nausea, loss of appetite, vomiting, diarrhea, and partial or complete obstruction of the small intestine (Dold & Holland, 2011; Guerrant et al., 2011a). The nutritional effects on the host are severe, and STH infections

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contribute to: protein energy malnutrition, deficiencies in Vitamin A and C, decreased fat and certain sugars absorption, and lactose intolerance. Infection with *A. lumbricoides* also causes stunted or poor growth and impairment of cognitive development in children (Guerrant et al., 2011a; Scott, 2008).

Individuals with heavy infections of *T. trichiura* may experience diarrhea with loose, frequent, painful, and watery stools that may also contain blood and have an acrid smell (Bethony et al., 2006; CDC, 2013b; Guerrant et al., 2011c). Severe inflammation in the colon can result in colitis and dysentery (Bethony et al., 2006). Furthermore, rectal prolapse can occur, as well as severe anemia and impaired growth and cognitive development. (Bethony et al., 2006; CDC, 2013b; Guerrant et al., 2011c). Infection with hookworm can cause intestinal blood loss that can result in iron-deficiency anemia as well as chronic protein loss. Other symptoms include: dermatitis at the site of larva penetration, urticarial rash, pneumonitis, wheezing, dyspnea, pharyngeal irritation, hoarseness, nonproductive cough, nausea, vomiting, mild abdominal pain, anorexia, poor concentration, and fatigue (Bethony et al., 2006; CDC, 2013b; Guerrant et al., 2011b).

2.2.3 Factors Associated with Infection and Control Strategies

Numerous factors have been determined to be associated with an increased risk of infection with STHs. Different environmental, behavioral, and social predictors have shown to increase *Ascaris* infection, although they vary across studies and population groups. These factors include: open defecation, inadequate water supply, poverty, crowding, poor education of mothers, poor nutritional status, use of biosolids as fertilizers, not washing hands before eating, geophagy, and owning pigs (Scott, 2008). Colston and Saboyá (2013) state that residence in a wet and tropical climate, lack of education, poor living conditions, lack of safe water, inadequate disposal of human excreta, and working in agriculture, are determinants of STH infection. Age is also considered a risk factor, with preschool (1 through 4 years of age) and school-aged

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children (5 through 14) most at risk, since intensity of infection peaks during childhood. Halpenny, Paller, Koski, Valdés, and Scott (2013) referenced studies supporting predisposition to heavy worm infection, poor hygiene, not wearing shoes, and geophagy, as increasing an individual's likelihood of STH infection. Household factors included were household crowding, limited latrine access, and low maternal education. Furthermore, it has been demonstrated that STH infections cluster at the regional and national level and have been associated with soil type, vegetation cover, altitude, rainfall, and temperature.

A study carried out in El Salvador found potential risk factors for soil-transmitted helminthiasis to include: being of lower socioeconomic status, having dirt floors, owning pigs, working in agriculture, working in fields where household biosolids were used, and children between 6 and 12 years of age (Corrales et al., 2006). In another study, carried out in Ecuador, a birth cohort was followed during the first three years of life. Risk factors for this age group were determined to be: being lower in birth order, having an Afro-Ecuadorian mother, which is a marginalized ethnic group in Ecuador, or having a mother young in age or illiterate, being of low socioeconomic status, living in overcrowded conditions, living in urban environments, and having a father or other household member with an STH infection after birth of the child. The study also pointed out that mothers with STH infections while pregnant, and particularly those with moderate to high worm burdens of *Ascaris* and *Trichuris*, created a greater risk of infection for their children during the first three years of life. Mothers with an STH infection during the child's first year of life was also associated as being a greater risk factor. A child was found to be 11.6 more times likely to be infected with an STH if the mother had a moderate to heavy infection (Menzies et al., 2014).

Furthermore, a study carried out in Panama, in the non-coastal part of the Comarca Ngäbe-Buglé, examined regional, household, and individual factors related to reinfection of preschool aged children after administration of deworming medication. Clusters of infection

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were found to exist within this region of extreme poverty, and it was determined that transmission and reinfection were affected by both household and individual factors. In this study, stunted children were found to become more heavily reinfected after treatment with deworming medication (meaning they had a higher reinfection intensity). These children are thought to be more susceptible to *Ascaris* and hookworm infection. In addition, predisposition to heavy STH infection was examined. High levels of eggs per gram of feces (epg) before treatment influenced the intensity of reinfection with *Ascaris* and hookworm. It is understood that a genetic component, related to immunity, predispositions an individual to high worm loads. Household factors, such as low asset-based household wealth indices (number of family members per room, construction materials of floors and walls, and access to running water and a latrine) and low maternal education level, were associated with higher reinfection intensity with *Ascaris* (Halpenny et al., 2013).

Various control efforts have shown to be effective in reducing the morbidity of STH infection as well as the overall prevalence of soil-transmitted helminthiasis. The WHO's primary control strategy is through deworming, or preventive chemotherapy. Morbidity can be controlled, or at least reduced, through periodic treatment to those at risk by reducing the intensity of the infection. At-risk groups are defined as: "preschool children; school-aged children; women of childbearing age (including pregnant women in their second and third trimesters and breastfeeding women); and adults in certain high-risk occupations, such as tea-pickers or miners" (WHO, 2014). The current recommendation is to provide the deworming medication to all people at risk in an area where STH infection is endemic. At least one dose of either albendazole (400 mg) or mebendazole (500 mg) is recommended once a year in areas where STH prevalence is between 20% and 50%, and twice a year in areas with prevalence over 50%. The current treatment target is to provide 75% of children who are at risk for STH infection with deworming medications. The WHO also recommends health and hygiene

education and access to sanitation, in addition to deworming, for helminth control (WHO, 2014). Deworming programs can be incorporated into other health interventions in order to reach the target population. This can be done through administering deworming medication during vaccination campaigns, or along with Vitamin A distribution programs and other health programs, such as those focused on reproductive health. An additional way to integrate deworming is through the school system. This avenue can be beneficial because many times a health or hygiene education component is included, or possible improvements in water and sanitation are built into the deworming campaign. Deworming has a direct and positive effect on school attendance and physical and cognitive development (PAHO, 2007).

Various studies and meta-analyses have been carried out demonstrating the effectiveness of deworming and water, sanitation, and hygiene (WASH) related strategies for the control of soil-transmitted helminthiasis. Results of WASH interventions include decreased prevalence and transmission of STH infection, as well as positive behavioral changes, such as increased hand washing (Gyorkos, Maheu-Giroux, Blouin, & Casapia, 2013; Strunz et al., 2014; Ziegelbaurer et al., 2012). Preventive chemotherapy is a global strategy to control morbidity of soil-transmitted helminthiasis, but deworming does not prevent reinfection. Integrated control approaches are necessary in order to prevent or interrupt reinfection and eliminate helminthiasis at the local level (Ziegelbaurer et al., 2012). Providing access to improved sanitation is the key factor in an integrated control program (IDB et al., 2011; Ziegelbaurer et al., 2012), and deworming (mass drug administration) should be combined with improved access to sanitation, clean water, and health and hygiene education (Bethony et al., 2006; IDB et al., 2011; Ziegelbaurer et al., 2012), in addition to investments in economic development in populations that are at risk and endemic for STH infection (IDB et al., 2011). Moreover, improved surveillance and mapping of STH prevalence and intensity are needed in order to better determine the populations at risk, where to best focus efforts, and how to better guide control

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strategies (Saboyá, Catalá, Nicholls, & Ault, 2013). The emergence of resistance to antihelmintics with widespread use is a concern, and efforts also need to be placed on the development of new medications for helminth control programs (Bethony et al., 2006; Guerrant et al., 2011a; Guerrant et al., 2011b).

2.3 *Ascaris* and Sanitation

2.3.1 *Ascaris* and Implications for Wastewater Reuse and Land Application of Biosolids

Increasing access to improved sanitation facilities is an important part of the UN Millennium Development Goals (U.N., n.d.) and is necessary to reduce the global prevalence of soil-transmitted helmintiasis and diarrheal disease (WHO, 2002). Globally, 2.5 billion people still lack access to an improved form of sanitation, with the majority of these people residing in the developing world (WHO & UNICEF, 2013). Many factors affect which sanitation technology is most appropriate for a population, and financial reasons play a large role. In industrialized countries (and in some large cities of developing countries), highly mechanized systems are used to treat excreta and are very expensive. These systems also require large amounts of water and are not feasible in areas suffering from water scarcity or poor availability of freshwater sources (Mara, 2003; International Water Management Institute [IWMI] & International Development Research Centre [IDRC], 2010). For sustainable treatment of excreta in the developing world, sanitation systems need to: consider the cost (should be low in cost for both the initial construction and operation and maintenance); be simple to operate and maintain; use low amounts energy and chemicals, or none at all; require less land (unless large areas are available for treatment systems such as wastewater stabilization systems/ponds); and produce a good quality effluent (if applicable) and low amounts of sludge (Mara, 2003).

One way to ensure that sanitation systems are more sustainable is to incorporate the concept of resource recovery and beneficial reuse into the design and operation. Excreta, wastewater, and sludge contain numerous nutrients, such as: phosphorus, nitrogen, calcium,

and magnesium. These nutrients can be recovered and used on agricultural crops, through irrigation with wastewater and land application of sludge/biosolids as a soil amendment. For centuries, land application of excreta, wastewater, and sludge has been widely practiced globally (IWMI & IDRC, 2010).

The beneficial aspect of land application for agricultural purposes can quickly become detrimental if the pathogens contained in feces are not sufficiently inactivated or removed during sanitation treatment. *A. lumbricoides* is very persistent in the environment and not easily inactivated (Feachem et al., 1983; WHO, 2006). *Ascaris* ova are used as an indicator for treatment effectiveness due to their thick shells and high resistance to stressors (Environmental Protection Agency [EPA], 2013). The EPA and the WHO have created guidelines for reducing the risk of infection from wastewater and sludge used for land application. The EPA's land requirement for biosolids is <1 viable helminth ova/ 4 grams of total solids (dry weight) (EPA, 1994). The WHO guideline for verification monitoring in treated feces and fecal sludge is <1 helminth egg/ gram total solids. For wastewater used in agriculture, the value is set at ≤1 helminth egg/ liter of wastewater. If children under 15 are at risk for exposure to irrigation with wastewater, then this value is reduced to ≤ 0.1 helminth egg/ liter (WHO, 2006).

Sanitation type can be grouped into either on-site (decentralized) or off-site (centralized or semi-centralized) technologies. Dry sanitation, or ecological sanitation, is a viable option of on-site sanitation. It separates urine from feces, and the urine and stabilized compost (biosolids) can be used as fertilizer. Composting latrines are an example of ecological sanitation and are a feasible alternative to water-based sewer systems. In areas where off-site disposal is needed or desired, wastewater stabilization ponds (WSPs) are an effective method for treating wastewater, if sufficient land is available (WHO, 2002) . WSPs incorporate the concept of resource recovery, and their effluent is frequently used for irrigation of crops and the sludge as a soil amendment (Mara, 2003).

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WSPs are widely used throughout the world and are seen as a first choice for wastewater treatment in numerous locations. They are a relatively inexpensive, simple, and low-maintenance process for wastewater treatment, and are ideal for small communities, although they are used in larger cities as well (Kefalla, Harrerimana & Vasel, 2013; Nelson, 2003; Verbyla et al., 2013). WSPs are considered an appropriate technology for developing countries and rural areas within developing countries (Stott, May & Mara, 2003). The ponds are large, shallow basins and can be made up of three different types; anaerobic, facultative, and maturation ponds, set up in series.

Treatment occurs via sedimentation and by biological and chemical processes. A layer of sludge develops at the bottom of the ponds, with the greatest amount of sludge accumulating in the anaerobic pond or the first pond in the system. Helminth eggs are removed from the wastewater during sedimentation. The majority are usually removed in the anaerobic pond (or first pond) and additional eggs can be removed in the facultative and maturation ponds (Konaté, 2013). Nearly complete removal of helminth eggs from the end effluent can be achieved if the WSPs are operated and designed correctly (Nelson, 2003). Although stabilization occurs in the ponds, many pathogens are still found within the sludge, particularly helminth eggs, and higher levels of *Ascaris* eggs are recorded in ponds in communities with higher prevalence rates of Ascariasis (Nelson, 2003; Nelson, Jiménez Cisneros, Tchobanoglus, & Darby, 2004).

Removal of sludge from the WSPs is required on a periodic basis, but sludge management is usually not a primary focus when designing a pond system. More information is necessary to ensure safe and effective management of sludge, due to the high levels of viable pathogens present. Research is lacking in understanding the different characteristics of the sludge, sludge accumulation and distribution within ponds, and pathogen concentrations and viability (Nelson et al., 2004). Parasite accumulation and survival is important to determine if further treatment of the sludge is needed after removal from the WSPs (Konaté et al., 2013).

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In a study carried out in Bolivia with a three pond system, complete removal of *Ascaris* was found in the system effluent; however, *Taenia* eggs were present (Verbyla et al., 2013). A study done in Brazil demonstrated complete removal of *Ascaris* from a five pond system. It was determined that an average of 94.6% of parasite eggs were removed in the anaerobic pond, and this average increased to 99.6% in the facultative pond. Complete removal did not occur until the second maturation pond, and the effluent from the third maturation pond was also *Ascaris*-free (Stott et al., 2003). In Burkina Faso, there was 100% removal of helminthes in the effluent during June to February. During the hotter months (March to May), the removal decreased (1-3 eggs per liter were observed). In the anaerobic pond, higher concentrations of eggs were observed in the sludge, which is where the greatest accumulation of sludge had occurred. An average of 995 eggs per gram total solids were found at the inlet, 795 in the middle, and 254 eggs per gram total solids at the outlet. Even after seven years of operation, the concentrations remained high. The inlet contained an average of 420 eggs per gram total solids, the middle 344, and the outlet 105 eggs per gram total solids. Viability was calculated according to sludge depth, measuring from the bottom up. The deepest sludge (at 0 cm) had zero viability of helminth eggs, and the top layer of the sludge recorded the highest viability. For *A. lumbricoides* at 0 cm, there were 0 viable eggs per gram total solids and 19 nonviable eggs per gram total solids. At 120 cm from the bottom, there were 43 viable eggs per gram total solids and 85 nonviable eggs. Closest to the surface at 180 cm (from the bottom), 305 viable eggs per gram total solids were recorded and 345 nonviable eggs found (Konaté, 2013).

2.3.2 *Ascaris* and Ecological Toilets

Composting latrines are a form of dry/ecological sanitation that convert feces into a soil amendment, with beneficial reuse of the compost produced for application on crops. There are different designs of composting latrines, but most have two chambers and use urine-diversion. These are known as Double-Vault Urine-Diversion (DVUD) composting latrines. Figure 5 is a

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drawing of the latrine structure and chambers. The toilet seat is designed in a way that feces pass directly into the chamber through one of two holes in the seat. Urine passes through the other hole in the seat and is diverted out through a small tube, either into a soak pit in the ground or some form of storage container for use as fertilizer. Figure 6 contains a picture with an aerial view of a latrine seat in Panama, demonstrating feces and urine separation in the design. Composting latrines are above-ground structures, and can be built in areas where other traditional forms of latrines (i.e. the common pit latrine) cannot. They can be built in areas with high water tables, flooding issues, and in areas close to surface water. Composting latrines are a dry sanitation method, and therefore do not require water. This feature is appropriate in areas suffering from water scarcity. Use of desiccants are required to assist in the composting process, and also to keep odors low. The latrines are typically constructed out of concrete and designed with two chambers (vaults), so that one side can be in use while the other is storing feces for a time period of at least one year or more. Each latrine has two doors on the backside for access into each of the chambers. These doors should be designed in a way that they can be easily opened and properly sealed shut. An additional form of dry sanitation is the solar latrine. These latrines differ from composting latrines because they use a solar heater that concentrates solar energy to increase the temperature, rate of evaporation, and dehydration of the contents inside the chamber. Some prototypes have one large chamber while others consist of two chambers (Cruz Espinoza, 2010).

The operation of composting latrines requires more effort than other forms of latrines, such as the pit latrine. One major difference is that desiccants must be used in composting latrines after each defecation. Also, the contents are mixed throughout the process and eventually harvested from the chambers and handled for land application. Before using

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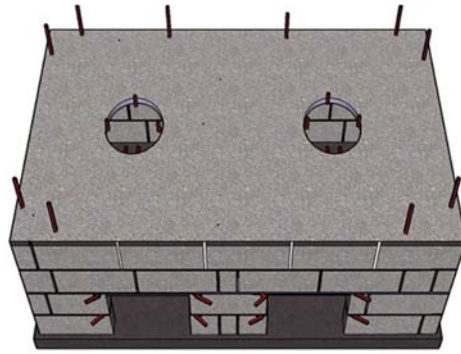
Figure 5. Example of the Base Structure of a Composting Latrine in Panama (Peace Corps Panama, 2013).

Figure 6. Aerial View of Composting Latrine Toilet Seat with Separation for Urine and Feces (Peace Corps Panama, 2010).

one of the chambers, the inside should be primed with dry organic material, such as dried grass, thick enough to at least cover the bottom of the chamber. Once the bottom is primed, the chamber can be put into use. After each defecation, dry materials, or desiccants must be added to the chamber to cover the fresh feces (Mihelcic et al., 2009). Common desiccants include: wood ash, sawdust, rice husks, lime, dry soil, dry leaves, and dry cut grass (Mihelcic et al., 2009; Peace Corps Panama, 2010). The dry materials should be stored inside of the latrine next to the toilet seat for easy use. Caution is required to ensure that the dry materials are only thrown into the hole designed for the feces. Dry materials should not enter the section for urine,

and it is necessary to prevent the urine duct from becoming clogged with desiccant. After the dry materials have been added to the latrine, the seat cover should be closed to prevent insects from entering. It is recommended to mix the pile periodically throughout use. The other toilet seat that corresponds to the chamber not in use should be sealed shut so that no one uses that side of the latrine until it is ready. Only one chamber should be at used a time (Mihelcic, 2009; Peace Corps Panama, 2010).

Once the chamber in use is full, additional dry materials should be added and the contents mixed. It should be properly sealed for a period of at least one year (but longer is recommended, if possible) and then the contents harvested (Mihelcic et al., 2009). A secondary treatment method should occur, such as placing the compost on a zinc sheet and setting it out in the sun for a week (preferably during the dry season). The compost can then be buried or mixed into soil surrounding the base of ornamental plants or fruit trees. It should not be used on crops that are eaten raw, grown under the ground (root vegetables), or on any low-lying fruits and vegetables (lettuce, strawberries, etc.) (Mehl et al, 2011; Mihelcic et al., 2009).

The composting process, in general, requires a number of factors for aerobic decomposition and pathogen destruction to take place. It occurs when organic waste is broken down biochemically, in the presence of oxygen, and produces a stable, pathogen-free, humus-like product. For this composting process to occur, the following environmental factors are required: temperature, pH, carbon to nitrogen (C/N) ratio, moisture content, aeration, and particle size (Mihelcic, 2009). If the necessary parameters are met, then the temperature in the pile will increase from ambient air temperature to mesophilic temperatures (with mesophilic microorganisms most active). From there the temperature in the pile will reach thermophilic temperatures (40˚C to 70˚C), which is when thermophilic microorganisms are present. It is during this stage that pathogens are destroyed and maximum stabilization and degradation

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occurs. Temperatures then return to mesophilic levels and the curing (cooling) stage begins

(Metcalf & Eddy, Inc., 2003). The required parameters for composting are found in Table 9.

Table 9. Requirements for Composting (Adapted from Wang, Tay, Lee Tay, & Hung, 2010)

Parameter	Value
pH	6-9
C:N Ratio	25-35:1
Moisture Content	40-60%
Temperature	45-70˚C
Particle Size	10-50 mm
Aeration/Mixing	Periodic mixing required, frequency and rate vary by pile

Schönning and Stentström (2004), state that although the composting process is a

viable treatment for fecal matter, they do not recommend urine-diverting composting latrines as

a primary treatment method. They found that many latrines do not reach thermophilic

temperatures and do not have the correct parameters to undergo aerobic decomposition.

Therefore, the latrine contents does not go through the composting process. Instead, the

authors state that it is "storage and anaerobic putrification, desiccation, or alkalization that

occurs" (Schönning and Stentström, 2004). In order to inactivate pathogens in feces within

composting latrines (when aerobic decomposition does not occur), other methods can be used.

One such example is to increase storage time based on temperature. Most composting latrines

remain near ambient temperatures, so this would not be an effective method for pathogen

destruction in the majority of latrines, since it takes a year at 42˚C to destroy all pathogens,

including *Ascaris* (Mehl et al., 2011)*. Ascaris* can be considered an indicator for compost quality

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because the time-temperature requirements for complete inactivation are the most stringent (Feachem et al., 1983).

An additional method for pathogen destruction when aerobic decomposition is not possible is by alkalization and desiccation (dehydration). Alkalization occurs by adding a considerable amount of desiccant to the latrine contents, such as lime and/or wood ash, in order to elevate pH. A pH greater than 9 is needed to kill most pathogens (but not *Ascaris*) (Mehl et al., 2011). Dehydration can be used to lower the moisture content to below 25%. The moisture is lowered by adding enough dry material, such as sawdust, wood ash, or rice husks to the biosolids (Stockholm Environment Institute, 2004). Lowering the moisture content below 30-40% kills off most pathogens, but *Ascaris* is very tolerant to desiccation and has been found to survive even at 5% moisture content. Lime can also be added to lower the moisture content, while at the same time increasing pH, allowing for both dehydration and alkalization to occur concurrently (Nordin, 2010). A study carried out in El Salvador in 2003 demonstrated that pH alone played a minimal role in the inactivation of *Ascaris* in DVUDs and solar latrines, but paired with high temperature, inactivation occurred much more rapidly and was significant (pH ≥ 11, temperature ≥ 36˚C, and average moisture content 37%). While, pH alone played a more significant role in the inactivation of bacterial and coliform indicators in the DVUD composting latrines. In addition, when peak temperature reached 44˚C in the solar latrines, temperature alone was significant for inactivation. The authors mention that a similar trend was seen in a study carried out in Vietnam. Elevated pH (>10) was the most significant factor for inactivation of bacterial coliforms, while elevated pH and temperature combined were the most significant factors for inactivation of *Ascaris* in the latrines (both composting and solar) (Moe & Izurieta, 2003).

The 2003 El Salvador study also showed that storage time was significant for composting latrines only. It is proposed that this was due to the much longer average storage

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time in the composting latrines of 306 days (range 80-702 days) as compared to solar latrines (26 days, range 0-90 days). These DVUD composting latrines had an average temperature of 31˚C (range 22-36˚C), an average peak temperature only 1˚C above ambient temperature (31˚C), average moisture content of 37% (range 15-96%), and average pH of 9.6 (range 6.2 and 13.0). The solar latrines included in this study had an average temperature of 30˚C (range 23 to 37˚C), average peak temperature of 37˚C (30-44˚C), moisture content of 31% (range 3-77%), and an average pH of 9.9, (range 7.1 to 12.9). Only 59% of the DVUD latrines sampled produced biosolids with 1 helminth egg per 4 grams of total solids. The most recent model of solar latrines were much more effective than the two earlier prototypes used in El Salvador because they had a higher peak temperature (42˚C), pH (10.3), and storage time (31 days). This later model produced 100% inactivation of *Ascaris* and also contained lower levels of bacterial coliforms. 75% of the latrines of this prototype were within the levels needed for Class A Biosolids for coliforms. The authors recommend the promotion of the double chambered solar latrine (most recent prototype) as an effective sanitation system in El Salvador, provided that the solar box receives plenty of sun exposure, and the latrine contents receive ample amounts of pH-elevating desiccants (lime or lime/wood ash) and longer storage time. In addition, for any dry toilet, they recommend the use of higher pH desiccants since none of the latrines tested had undergone aerobic decomposition. (Moe et al., 2003).

A follow-up study was carried out in the same communities in El Salvador in 2006. This study was different because it examined the impact of various dry latrines on the prevalence of helminth and protozoa infections, while accounting for individual and household factors. The results showed that DVUD composting latrines were associated with a higher prevalence of *Ascaris* (11.5 times greater) and *Trichuris* (7.1 times greater) than other latrine types. The solar latrines were associated with a lower prevalence of these infections when compared with the DVUD composting latrines. The authors recommended the use of solar latrines over

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composting latrines, and the burying of biosolids after harvesting from the chambers because burying was associated with the lowest prevalence of infection (Corrales et al., 2006).

To build upon the information learned from the two studies carried out in El Salvador, Cruz Espinoza (2010) and Cruz et al. (2012b) carried out laboratory experiments to simulate the parameters of a solar latrine in El Salvador. These experiments were done in order to test the inactivation of *Ascaris suum* in amended feces, with and without the addition of urea. The laboratory experiment also expanded upon previous research completed by Nordin, Nyberg, and Vinnerås (2009a) that demonstrated *Ascaris* inactivation by ammonia in wastewater sludge with a high moisture content. Cruz's study used a lower moisture content instead; similar to what was found, on average, in the solar latrines in the field. Fresh human feces were collected and additives of dirt and lime were added. The amended feces were separated into three piles and 1% urea was mixed into one, and 2% urea into another. The third pile did not receive urea and served as the control. *A. suum* eggs were placed in small nylon bags and inserted into the amended feces. Duplicates were placed at 28°C, 35°C, 40°C, and 45°C, and placed in the dark for 56 days. The minimum starting pH was 8.3 with a moisture content of 27.5% (wet based). The samples were pulled out of the amended feces over the 56 day period to determine the effect of the urea concentration, treatment duration, and temperature on the viability of the eggs. The results, related to the samples treated with urea, and their significance, will be discussed in the next section (Section 2.3.3 Inactivation of *Ascaris* by Urea). The control (0% urea) demonstrated 100% inactivation in 21 days of storage at 35°C, 7 days at 40°C, and 24 hours at 45°C. By Day 56, 100% inactivation was still not observed for storage at 28°C without urea. The significance of this demonstrates that at 28°C, and initial conditions of pH 8.3 and a moisture content 27.5% (conditions similar to what is found in the field), complete inactivation of *Ascaris* ova will not occur unless urea is added. The laboratory experiments demonstrated that

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temperatures need to reach at least 35°C for 21 days at these parameters for complete inactivation to occur (Cruz Espinoza, 2010; Cruz et al., 2012b).

Mehl et al. (2011) carried out a field study in Panama in Bocas del Toro Province beginning in 2002. All of the sites included in the study were indigenous Ngäbe communities with DVUD composting latrines constructed and in use. Temperature, pH, use of desiccant, and moisture were measured in each of the latrines. Grab samples were taken from 5 latrines to compare field results to lab results. The most common desiccants observed to be used were sawdust and wood ash. Other desiccants used in much smaller amounts were dry grass or leaves, dry dirt or sand, rice and coffee husks. The grab samples mentioned were taken from latrines that had been closed for at least 6 months, with the exception of one, which had only been sealed for 4 months.   This last latrine was included because it had a higher pH than the rest. Samples of the biosolids within the latrine chambers were also collected and analyzed for pathogen content.

All five of the grab samples contained *A. lumbricoides* eggs. Other pathogens found within the biosolids included *Trichuris*, *Taenia solium*, *Entamoeba*, and total coliforms. These pathogens were present even after the recommended storage time of 6 months. In addition, the average recorded temperature for the 144 latrines sampled, was 29.5°C. The ambient daytime high for the region is 29°C. These results alone, support other results discussed in the article, that on average, composting latrines do not undergo aerobic decomposition as designed, and therefore the required pathogen destruction in the biosolids does not occur, even after a 6 month storage period. However, the results of this study carried out in Panama do suggest that aerobic decomposition could possibly be attained in the majority of the latrines sampled. 65% of the latrines had near neutral pH, and 87% had sufficient moisture levels; however, the C/N ratios were similar to feces, meaning that not enough carbon (sawdust, rice husks, dry leaves, etc.) was added to promote aerobic decomposition. In addition, the researchers did not record

Table 10. Experimental Set-up and Results for Biosolids Treatment in the Field (Non-Urea Based Treatment)

Type of Experiment	Experimental Set-up	Results	Source
Field-El Salvador	Combination of DVUD composting latrines and three different prototypes of solar latrines; tested temperature, pH, storage time, moisture content, and pathogen analysis Treatment Method: Alkaline stabilization/dehydration; storage time	pH alone not sufficient to inactivate *Ascaris* (but sufficient for bacterial coliforms); high pH combined with high temperature significant for *Ascaris* inactivation (pH ≥ 11, temperature ≥ 36˚C, moisture content ~37%); temperature alone was significant in solar latrines with peak temperature of 44˚C; storage time significant in composting latrines for those with longer storage time plus higher pH and temp. Conclusion: newest prototype of solar latrines inactivated all *Ascaris* eggs and reached higher temperatures; only 59% of DVUD composting latrines produced acceptable product (those with the longer storage time and higher pH and temperature)	Moe et al., 2003
Field-Panama	144 DVUD composting latrines studied; 5 with grab samples from latrines stored for >6 months (except for one with < 6 months because had a higher pH) to test for pathogen content and to compare field results with lab results; temperature, pH, moisture content, storage time measured Treatment Method: Storage time (goal of aerobic decomposition)	Average temperature in chambers was 29.5˚C; ambient daytime high 29.5˚C; 65% of latrines with near neutral pH and 86% with sufficient moisture for aerobic decomposition; C/N ratio same as feces; *Ascaris* present in all grab samples even after 6 month storage Conclusion: storage at ambient temperature not sufficient to kill off *Ascaris* in 6 months; need 42˚C for 1 year for inactivation; however, with additional high carbon source and mixing, should be able to achieve aerobic decomposition and kill pathogens	Mehl et al., 2011
Field-El Salvador	Tested association between latrine type and prevalence of helminth and protozoan infection Treatment Method: Alkalization/dehydration; storage time	DVUD composting latrines significantly associated with *Ascaris* and *Trichuris* infection; solar latrines associated with lower prevalence when compared with DVUDs Conclusion: solar latrines recommended over composting latrines and burying biosolids after harvesting because burying was associated with the lowest risk of infection	Corrales et al., 2006

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whether mixing was occurring or the frequency of mixing. The recommendations are to instruct

users to add in additional high carbon materials along with the sawdust to increase the C/N ratio

and to also mix the pile. The pile should be mixed and turned to support thermophilic activity

and aerobic decomposition of the biosolids. Other recommendations are to instruct latrine users

to use desiccants that elevate the pH, such as wood ash and lime mixed with dirt, and thus

promote alkalization by increasing the pH to greater than 9, and dehydration (desiccation) by

reducing the moisture content to below 25% (as recommended by Moe and Izurieta, 2003).

Recommendations for secondary treatment of the biosolids should be carried out as discussed

in Mehl et al. (2011) and Mihelcic et al. (2009). Table 10 provides an overview of these studies

discussed in this section.

2.3.3 Inactivation of *Ascaris* by Urea

Composting latrines are a great idea theoretically, but in actuality a good majority of

them do not undergo aerobic decomposition, and pathogens are not killed off. Thus, the

biosolids are unsafe for handling and are advised not to be used on crops. Urea, $CO(NH_2)_2$, is a

common form of fertilizer and consists of 46% Nitrogen (N). It undergoes chemical reactions, in

the presence of water and urease, to form Ammonia (NH_3). Ammonia is documented as an

ovicide and has been shown to inactivate *Ascaris* eggs under specific conditions and

concentrations (McKinley, Parzen, & Mercado Gúzman, 2012). The chemical reactions that

occur are described in Figure 7. At high pH and temperature, the equilibrium between

ammonium (NH_4^+) and ammonia (NH_3) favors the formation of ammonia, so the chemical

reaction proceeds to the right, and ammonia predominates (Nordin, Ottoson, & Vinnerås,

2009b). Equation 2 of Figure 7 includes this chemical reaction.

Urea hydrolyzes to ammonia (aqueous) and then to ammonia gas. When this occurs,

Ascaris and other pathogens have the potential to be destroyed due to contact with the

ammonia. This has led researchers to carry out experiments using urea to inactivate pathogens

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in solution, feces, and sludge/biosolids (Cruz Espinoza, 2010; Cruz et al., 2012b; Malavade et al., 2012; McKinley et al., 2012; Nordin et al., 2009a; Pescon, Barrios, Jimenéz, & Nelson, 2009). Nordin et al. (2009a) conducted an experiment where the research team collected feces,

<center>Urease</center>

$$CO(NH_2)_2 \; + \; H^+ \; + \; 2H_2O \quad \rightarrow \quad 2NH_4^+ \; + \; HCO_3^- \qquad \text{(Equation 1: Hydrolysis)}$$

Urea   Ammonium

$$NH_4^+ \quad \rightarrow \quad NH_{3(d)} \; + \; H^+ \qquad \text{(Equation 2)}$$

Ammonium   Ammonia

$$NH_{3(d)} \quad \rightarrow \quad NH_{3(gas)} \qquad \text{(Equation 3: Volatilization)}$$
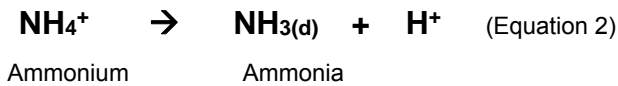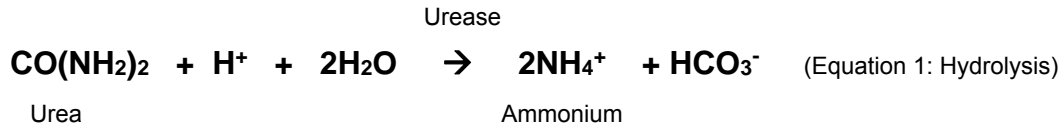
Figure 7. Chemical Reactions for Ammonia Volatilization from Urea (Adapted from Montana State University Extension & Washington State University Extension, 2007)

and added 0% and 1% urea to feces with and without ash amended. 2% urea was added only to the unamended feces (feces with no ash). *Ascaris suum* eggs were implanted into the collected feces and incubated in the dark for 35 days at 24˚C and 34 ˚C. For the 34 ˚C samples, by Day 4, no viable *Ascaris* eggs were found in those with the amended feces with ash and 1% urea, nor were any found in the feces with just 2% urea added. The feces amended with ash only and no urea had 2 viable eggs visible at Day 4. At Day 10, greater than 2,000 eggs were counted for each treatment group. For feces with 1% urea, no viable eggs were found, and by Day 31, no viable eggs were detected in the unamended feces. For the group at 24 ˚C, at Day 22, no viable eggs were found in the amended feces with ash and 1% urea. One viable egg was observed in feces with 2% urea on Day 35. The feces amended with ash had a 90% inactivation (+/- 4.5%), and those amended with 1% urea had 78% inactivation (+/- 6.5%). The unamended feces had 50% (+/-16%) inactivation. This study also suggested

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that inactivation rates might require a threshold NH_3 concentration of 20mM. Nordin et al, 2009a discussed similar findings in other literature reviewed (Nordin et al., 2009a).

Research carried out by Cruz Espinoza (2010) and Cruz et al. (2012b) was discussed previously. To expand on the results discussed, the authors concluded that inactivation of *A. suum* in solar latrines would be possible by adding 1% urea to a closed vault with a minimum temperature of 28°C , minimum moisture content of 27.5%, and initial pH of 8.3. All of the samples exposed to urea had 100% inactivation at 14 days at 28°C, 3 days at 35°C, and at 1 day for both 40°C and 45°C. Inactivation with urea occurred in a shorter period of time as compared to the Nordin et al., 2009a study. The Nordin et al. study was done with high moisture levels (83%-95%, wet based), whereas Cruz's study had a mean moisture content of 27%, wet based (Cruz Espinoza, 2010; Cruz et al., 2012b).

A pilot project was carried out in El Salvador, and the aim was to determine if urea would be appropriate for usage in solar latrines in the field. 1% urea was added to the biosolids harvested from the latrines for the treatment group, but was not added to the biosolids of the control group. The starting moisture content target was between 23% and 50%, and a pH between 8.0 and 10.0 for all samples. Biosolids were added to garbage bags for each sample. *A. suum* ova were placed in nylon bags and placed inside the garbage bags filled the treated or untreated biosolids. These biosolid-filled garbage bags were then placed into latrine chambers to simulate the internal latrine environment. A few additional garbage bags were placed outside of the chamber in the sun. Samples were pulled at Day 1, 2, 3, 4, and 5. 100% inactivation of *Ascaris* was not observed for any of the samples. This was attributed to a short treatment time, which resulted in an insufficient contact time for the ova with urea. Other studies with comparable parameters had treatment times of weeks to months. Another factor in this study was that temperature fluctuated more than anticipated, and peak temperatures were not sustained for prolonged periods of time. The studies previously discussed kept temperature

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Table 11. Experimental Set-up and Results for Biosolids Treatment in the Laboratory and in the Field Using Urea

Type of Experiment	Experimental Set-Up	Results	Source
Laboratory	Feces amended with 0% urea and ash, 1% urea and ash, 0% urea, 1% urea, and 2% urea; treatment time of 35 days at 24°C and 34 °C Treatment Method: Urea (ammonia)	34 °C samples: at Day 4, no viable *Ascaris* eggs were found in the amended feces with ash and 1% urea, nor in the feces with just 2% urea added. The ash amended feces only and no urea had 2 viable eggs visible at Day 4; at Day 10, feces with 1% urea had no viable eggs; by Day 31, no viable eggs were detected in the unamended feces 24 °C samples: at Day 22, no viable eggs were found in the amended feces with ash and 1% urea; by Day 35, one viable egg was observed in feces with 2% urea, and feces amended with ash had a 90% inactivation (+/- 4.5%) rate, those amended with 1% urea had 78% inactivation (+/- 6.5%), and the unamended feces had 50% (+/-16%) inactivation 24°C: feces with 2% urea, pH 8.9-9.1; 1% urea, pH 8.7-8.9; ash with 1% urea, pH 9.6-10.0; ash only, 9.7-10.5; 0% urea, pH 8.0-8.3 34 °C: 2% urea, pH 8.8-9.0; 1% urea, 8.7-8.8; ash with 1% urea, 12.7-12.8; ash only, 12.7-12.8; 0% urea, 8.1-8.3 Limitations: High levels of moisture; lab experiment instead of field experiment; temperature controlled for in experiment; in field, temperature will not remain constant for duration of experiment	Nordin et al., 2009a

Table 11. (Continued)

Laboratory	Feces amended with dirt and lime for a minimum starting pH of 8.3 and 27.5% moisture content (wet-based); samples with 0% urea (control), 1% urea, and 2% urea treated at 28°C, 35°C, 40°C, and 45°C; treatment time up to 56 days; lower moisture content used than Nordin et al., 2009a study Treatment Method: Urea (ammonia)	Control (0% Urea): 100% inactivation in 21 days at 35°C, 7 days at 40°C, and 24 hours at 45°C; at Day 56 100% inactivation still not observed at 28°C 1% Urea: at 28°C demonstrated complete inactivation at 14 days, 3 days at 35°C and 1 day at both 40 and 45°C; faster inactivation than Nordin et al., 2009a study Conclusions: at 28°C, initial pH of 8.3, and moisture content 27.5% (conditions similar to what are found in solar latrines in the field), complete inactivation of *Ascaris* ova will not occur unless urea is added; temperatures need to reach at least 35°C for 21 days at these parameters (no urea) for complete inactivation to occur Limitations: Laboratory experiment instead of field experiment; temperature controlled for in experiment; in field, temperature will not remain constant	Cruz Espinoza, 2010; Cruz et al., 2012b
Field- El Salvador	Created small reactors out of garbage bags and biosolids amended with lime and soil from solar latrines; added 1% urea to biosolids reactors for treatment group and 0% urea for control. Initial moisture content and pH manipulated to have values between 23% and 50% moisture content and a pH between 8 and 10; Samples pulled on days 1, 2, 3, 4, and 5 Treatment Method: Urea (ammonia)	Statistical analysis signified a weak, but positive association between urea concentration and adjusted inactivation rate, and a strong positive association between adjusted inactivation rate and duration of treatment; when consider ammonia gas concentration and duration of treatment together, a greater positive association of duration of treatment with adjusted inactivation rate was observed; a significant negative association was observed between duration of treatment time and viability (viability decreased with greater duration of treatment time) Limitations: Time was a limitation; samples were only treated with urea for 1-5 days, not sufficient for 100% inactivation of *Ascaris.* Also, temperature in the field fluctuated more than expected (previous studies in the lab controlled for temperature)	Malavade et al., 2012

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constant throughout the treatment time as this was controlled for in the lab experiments.
Statistical analysis was carried out for the experiment in El Salvador. Linear regression analysis
determined a weak, but positive association between urea concentration and adjusted
inactivation rate, and a strong positive association between adjusted inactivation rate and
duration of treatment. When ammonia gas concentration and duration of treatment were
considered together, there was a greater positive association of duration of treatment with
adjusted inactivation rate. Further analysis demonstrated a negative association (downward
trend), in that, as duration of treatment time increased, viability decreased (Malavde et al.,
2012). Table 11 provides an overview of the experiments discussed in this section using urea
to inactivate *Ascaris*.

CHAPTER 3: FIELD-BASED EXPERIMENTAL METHODS FOR THE INACTIVATION OF *ASCARIS* IN COMPOSTING LATRINES

3.1 Study Site Description

This field experiment is designed to take place in select rural indigenous communities in Panama along the Caribbean coast in the Bocas del Toro Province. These communities have high water tables and/or are located along rivers that flood easily during the rainy season, making pit latrines an inappropriate form of sanitation in these areas. A large number of these communities have Double-Vault Urine-Diverting (DVUD) composting latrines that were constructed by Peace Corps Volunteers or by the Panamanian Government. DVUD composting latrines are considered to be an improved form of sanitation; however, a study conducted by Mehl et al. (2011) (previously discussed) in this same region demonstrated these latrines to be ineffective in inactivating *A. lumbricoides* eggs in the Bocas del Toro Province. This proposed

Figure 8. Double-Vault Urine-Diverting Composting Latrine in the Comarca Emberá-Wounaan (Courtesy of Daniele Renzi)

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experiment would be carried out in a Ngäbe community that has numerous DVUD composting latrines in use. It is possible that a previous study site could be used if the latrines are still in use and maintained. Figures 8 and 9 provide examples of these latrines constructed in the field in Panama.

Figure 9. Double-Vault Urine-Diversion Composting Latrine in a Ngäbe Community in Bocas del Toro Province (Courtesy of Author)

3.2 Experimental Design

3.2.1 Introduction

The aim of this experiment is to determine if the application of urea to biosolids from composting latrines can be a viable option for inactivating *Ascaris suum* helminth eggs, and to determine the effect of treatment time (storage time) and solar heat (temperature) on inactivation. Also, the interaction of urea and solar heat will be determined. *A. suum* (from pigs) will be used as a model for *A. lumbricoides*, which is the infective agent in humans. The methods expand upon recent research carried out in the field in El Salvador using solar latrines, an alternate form of dry sanitation with urine diversion (Malavade et al., 2012), and experiments conducted in the laboratory (Cruz Espinoza, 2010; Cruz, Allanson, Kwa, Azizan, & Izurieta, 2012a; Cruz et al., 2012b).

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This experiment would require training on handling of biological agents. In addition, a local partner within Panama would need to be established that has biological laboratory capacities because the preparation of the nylon bags with *Ascaris* eggs, and the slides for microscopy, need to be carried out under a biological safety hood. Previous researchers in the Mehl et al. (2011) study partnered with the Natural Resources Laboratory at the Autonomous University of Chiriquí (UNACHI) in David, Panamá. The rest of the experiment can be carried out at the field site location as long as there is a reliable source of electricity. It is recommended to access a community close to the Pan-American Highway for a shorter transport time to and from David.

The proposed field experiment consists of preparing duplicate samples containing *A. suum* ova for inoculation into thirty small reactors filled with biosolids from composting latrines that are in use at the experimental location. The latrines chosen will be latrines that are properly maintained, that use a desiccant, and that contain sufficient biosolids to meet the need of the experiment. Desiccants commonly used in this region include: sawdust and wood ash. Figure 10 contains an example of desiccant stored next to the toilet seat of a latrine for easy application after defecation.

Figure 10. Examples of Desiccant Stored Next to Toilet for Application after Defecation (Courtesy of Danielle Renzi and Patricia Wilbur)

The thirty reactors will be set up into four groups based on the combination of two different variables. Group A_1 will include six reactors without application of urea or exposure to sunlight (solar heat), which will be abbreviated to (U- S-). These reactors will be considered the experimental controls. Group A_2 will also serve as a control, and will contain six reactors with the same conditions as Group A_1, (U- S-). Two sets of controls will be used to account for any equipment failure or other errors that could occur in the field and to ensure accuracy of control results, since the control data is what will be used to determine effectiveness of urea treatment, exposure to sunlight, and treatment time. The next three groups will serve as the intervention groups. Group B will include six reactors without application of urea, but with exposure to solar heat, abbreviated as (U- S+). Group C will consist of six reactors with application of urea and no exposure to solar heat, (U+ S-). Finally, Group D will have six reactors that include application of urea and exposure to solar heat, (U+ S+). Group A and C reactors will be placed within sealed chambers of composting latrines to avoid exposure to solar heat, and those with solar heat exposure (Groups B and D) will be placed just outside of the latrines containing the reactors from Groups A and C in order to receive natural sunlight. Figure 11 provides an overview of the experimental setup.

Sampling of the *Ascaris* ova from each reactor will occur over a period of 56 days, with sampling occurring on Day 7 (T_1), Day 14 (T_2), Day 21 (T_3), Day 28 (T_4), Day 42 (T_5), and Day 56 (T_6). Viability of the eggs would then be determined after a three-week period of incubation using standard microscopy. Temperature, moisture content, pH, and ammonia levels will be measured throughout. Statistical analysis will be carried out at the end using Microsoft Excel and SAS v.9.4 software to determine the significance of the results. These results will guide recommendations for the operation of DVUD composting latrines in Panama.
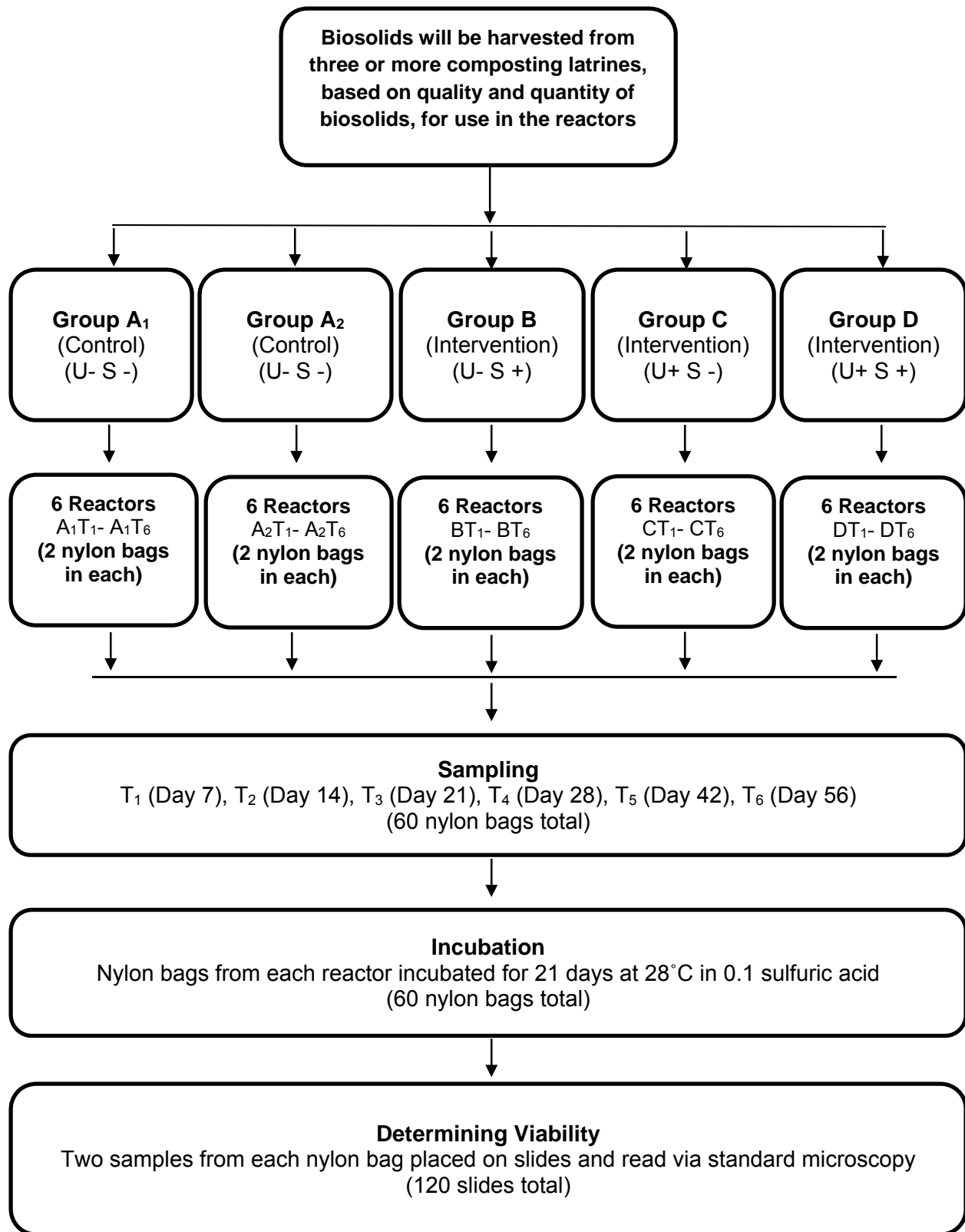
Figure 11. Overview of the Experimental Setup

3.2.2 Preparation of *Ascaris* Ova

In order to carry out this experiment, *A. suum* eggs are required. These eggs can be purchased from Excelsior Sentinel, Inc. (Ithaca, NY). They are stored at 4˚Celsius until they are ready to be used. The first step in the preparation of the ova is to create sixty nylon mesh bags that will each hold approximately 10,000 eggs. Nylon mesh with a 30 micron opening is to be cut 1.5" by 3" and sealed on the two sides, leaving the opening at the top unsealed. A bag sealer (8" Wide Electric Bag Sealer, A. Daigger & Company, Inc., Vernon Hills, Illinois) is used to seal the nylon bags. 200 µL of the *A. suum* stock solution (original concentration 50,000 eggs/mL) will be pipetted into each bag and the top sealed off. Fishing wire will be tied to each bag and a plastic identification tag (ID) will be attached to each string. The ID tag will include: the reactor number, which accounts for treatment time (day of sampling), such as T_1, T_2, etc., and location of reactor (i.e. A_1, A_2, B, C, or D); the time and date placed into the reactor; and type of intervention (i.e. U- S+), written with a permanent marker. Each bag will be stored in deionized (DI) water at 4˚Celsius until they are ready to be placed inside the reactors.

3.2.3 Preparation of Reactors

The experiment requires creating thirty different reactors using the biosolids from at least two different latrines and inoculating each reactor with two of the prepared nylon mesh bags filled with *A. suum* ova. The first step is to determine which latrines have sufficient biosolids in their chambers to meet the need of the experiment. These latrines must be latrines that are continuously in use or that contain a chamber filled with biosolids that was recently sealed, they must use some form of desiccant, and be properly maintained. The pH and moisture content of the biosolids removed from each latrine, for use in the reactors, must also be uniform in order to control for these two factors. This can be done by placing one or two large, clean, dry, plastic tarps down on the ground. The contents from the latrine chambers can be extracted through the use of a clean shovel. Those handling the biosolids must wear gloves or some other

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protective device to prevent feces from touching hands. In addition, at a minimum, long pants and sleeves should be worn, with closed-toed shoes to prevent contact of skin with feces. For this experiment, three-hundred pounds of biosolids are needed because each reactor will be filled with ten pounds of biosolids. Two or three piles of biosolids can be formed on top of the tarps, with approximately equal amounts of the biosolids added to each pile from each latrine. The piles should be thoroughly mixed using a shovel.

The next step, once the three piles have been sufficiently mixed, is to use soil pH and moisture meters in order to determine the pH and percent moisture content of the biosolids. It is necessary to keep these two variables constant throughout the experiment in order to determine if urea, temperature, and contact time have an effect on inactivation of the ova. Therefore, the pH and moisture content will need to be manipulated so that each reactor receives biosolids that are fairly uniform in this respect, and also to ensure that sufficient urease is present in the biosolids. Urease is found in human feces. There are a number of different bacteria present in the gastrointestinal tract and in fecal material that produce urease. Urease is also present in soil, and it is needed for urea to be converted to ammonia (Mobley, H.L.T., & Hausinger, R.P., 1989; Suzuki, K., Benno, Y., Mitsuoka, T., Takebe, S., Kobashi, K., & Hase, J., 1979; Wozny, M.A., Bryant, M.P., Holdeman, L.V., & Moore, W.E.C., 1997).

The goal is to bring the contents to a pH between 8.0 to 10.0, and a moisture content between 23% and 50%. If the pH is higher than 10, then sawdust and soil will be added to the biosolids and the pile will be mixed again with a shovel. Once the pile has been thoroughly mixed, the pH will be measured a second time. If the pH is still too high, then this process will be repeated until it falls between 8.0 and 10.0. If the pH is too low, then wood ash and/or lime will be added, and the same mixing process will be repeated until the pH falls within the required range. If the moisture content is below 23%, then water will be added and the biosolids will be mixed until the moisture content is between 23% and 50%. If the moisture content is higher

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than 50%, then sawdust and soil will be added until the moisture is within the correct range. Figure 12 includes an example of the contents of a latrine. The chamber includes feces, sawdust as desiccant, and used toilet paper.

Each of the reactors needs to be filled with ten pounds of the biosolids, adjusted for pH and moisture content. The first sets of reactors to be prepared are those in Groups A_1 (U- S-), A_2 (U- S-), and B (U- S+), since reactors in Groups C (U+ S-) and D (U+ S+) will be prepared differently to account for the addition of urea. There are six reactors (T_1 through T_6) for each Group. In order to weigh the reactors, a suspended balance with a weight of ten pounds will be hung over a tree. Each reactor, made of a thick black garbage bag, will be filled with biosolids and attached to a hook on the other end of the suspended balance. Additional biosolids will either be added or removed until the desired weight of ten pounds is achieved.

Figure 12. Biosolids Contents of a Latrine in a Ngäbe Community in Bocas del Toro Province, with Sawdust as the Primary Desiccant (Courtesy of Patricia Wilbur).

Before each garbage bag is sealed with duct tape, two holes will be punched out of the biosolids about halfway down and equally spaced apart using a 2-inch piece of PCV pipe. A nylon mesh bag containing the *Ascaris* eggs will be placed into each hole, and these holes will be filled in using the biosolids initially removed with the PCV pipe. It is important to maintain the

ID tags above the level of the biosolids for easy removal of the nylon mesh bags during the sampling phase of the experiment.

Temperature will be recorded throughout the entire treatment period, from Day 0 until Day 56. This will be done by using Track-It Temperature Data Loggers (MicroDAQ.com, Inc., Contoocook, New Hampshire) that contain a microchip for recording temperature. Two temperature loggers will be placed into all T_5 and T_6 reactors, and will be set to record every 10 minutes once bags are sealed for the treatment phase of the experiment. To protect the temperature loggers from fecal contamination each logger will be placed into a single zip lock bag and left opened, but implanted sufficiently enough into the biosolids. In addition, the pH and the moisture content will be measured and recorded before each bag is sealed on Day 0 of the experiment. Each reactor will have its own ID tag on the outside of the garbage bag containing the same information as the ID tags on the individual nylon mesh bags.

All of the Group A_1 and A_2 reactors (six for each group) will be placed into two separate latrine chambers. The first chamber will be known as Experimental Location A_1 (EL-A_1) and will contain only Group A_1 bags (A_1T_1- A_1T_6). The second chamber, EL-A_2, will contain all A_2 bags (A_2T_1- A_2T_6). Group B bags (BT_1- BT_6), will be placed just outside of EL-A_1 on the ground and in the sunlight with a fence surrounding them to prevent animals and small children from interfering with the experiment. This location will be EL-B. Groups C and D bags will contain urea, with Group C reactors placed within a third latrine chamber, EL-C, and Group D bags placed on the ground in EL-D, just outside of EL-A_2. Before the doors of the latrine chambers are closed, two temperature loggers will be placed on top of the remaining latrine contents, spaced equally apart. Any gaps between the door and latrine walls will be filled in with rubber tubing. This is done to replicate conditions in the field (a closed chamber, with sealed doors). Two temperature loggers will be placed outside alongside the reactors in both EL-B and EL-D.

Group C and D reactors require the addition of urea, and it will be added to each bag according to weight. The concentration of urea used will be 1% weight/weight, which is 1 gram of urea for 99 grams biosolids (wet). This means that each reactor will contain 10 pounds of biosolids and 0.1 pounds of urea, which also translates to 4.55 kilograms of biosolids and 45.5 grams of urea. The urea will be weighed in grams, using a small scale. Each bag will be filled with the 10 pounds of biosolids, and the pH and moisture content will be recorded for Day 0. The urea will be mixed in using a wooden stick, being careful to keep the opening of the bag closed as much as possible. Very quickly, but carefully, two holes will be punched out of the biosolids, and two nylon mesh bags with *Ascaris* will be inserted and covered back up in the same fashion as the reactors without urea. The top of the bags will be grasped together and then sealed with duct tape at the top, reducing the dead space as much as possible, but leaving room for this grasped section of the garbage to expand open a little bit once the ammonia begins to volatize. Temperature loggers will be placed in reactors CT_5, CT_6, DT_5, and DT_6. The preparation of the reactors marks Day 0 of the experiment, and the next steps include the treatment phase T_1 through T_6, and concurrent sampling at corresponding time intervals for processing the samples for the incubation phase.

3.3 Sampling and Incubation Phase

3.3.1 Detailed Sampling and Incubation Methods

Sampling will occur over a period 56 days, and the nylon mesh bags will be extracted from the reactors on specific days as noted below. The experiment was set up to contain two samples in each reactor, which serve as duplicates. There are to be 30 reactors, and therefore 60 total samples. Duplicates will be pulled out on Treatment Day 7 (T_1), Day 14 (T_2), Day 21 (T_3), Day 28 (T_4), Day 42 (T_5), and Day 56 (T_6) for each of the 5 groups of reactors (A_1, A_2, B, C, and D). This means that on any given extraction day, there would be 10 samples to process for incubation. At the time of extraction, the date and time will be recorded on the data sheet. In

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addition, the pH and moisture content of the biosolids, and the levels of ammonia gas, will be measured and recorded, being careful to not let too much of the ammonia gas escape through the opening in the garbage bag. Measuring the ammonia levels should be done first. Once all of the measurements are complete, the nylon mesh bags will be removed from the reactors and placed in zip lock bags and carried back to the laboratory in a cooler at ambient temperature. The temperatures will be downloaded onto a computer at the end of the experiment and recorded.

In the laboratory, the nylon bags will be processed for the three-week incubation phase. The first step involves rinsing the nylon bags twice with DI water to remove any residue. The bags are then placed in test tubes with 20 mL of 0.1N sulfuric acid, and the caps lightly screwed on. These test tubes are incubated at room temperature (approximately 28˚C) for 21 days. Every 48 hours the level of the sulfuric acid will be checked, and DI water added to bring the solution back up to original levels (as marked with a black marker on the outside of the test tubes).

The T_1 bags are pulled from the reactors on Treatment Day 7, which corresponds to Incubation Day 0. On this day, all of the T_1 bags will be placed into incubation with sulfuric acid for three weeks. They will be removed from incubation and read for viability on Incubation Day 21. All of the T_2 bags will be placed into incubation on Incubation Day 7 and read on Incubation Day 28. The rest of the bags will follow the same procedure. T_3 bags will be read on Incubation Day 35, T_4 bags on Incubation Day 42, T_5 bags on Incubation Day 56, and all T_6 bags will be read on Incubation Day 70.

3.4 Determining Viability of *Ascaris* Ova

3.4.1 Ova Viability Counting

To determine the viability of the eggs in the nylon bags, standard ova viability counting procedures will be followed as discussed in detail elsewhere (Cruz Espinoza, 2010; Cruz et al.,

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2012a; Cruz et al., 2012b; Malavade et al. 2012). Viability will be determined by using a standard microscope. After each sample undergoes incubation for 21 days, the test tube will be opened up and the nylon mesh bag removed. The mesh bag will be carefully cut across the top, and the eggs will be washed directly into a petri dish using approximately 2mL of the sulfuric acid. A sample of 25 µL of the sulfuric acid/ova solution will be pipetted from the petri dish onto a microscope slide. A cover sheet will carefully be placed on top of the slide and the eggs will be examined for viability under the microscope at 40X and 100X magnification, counting 400 eggs total per sample. A second sample from the same nylon mesh bag will be prepared and counted, which will serve as a duplicate. This same process will occur for every nylon mesh bag that was part of the treatment phase. Sixty nylon mesh bags were used for the experiment, thus 120 samples will need to be read under the microscope. Initial viability of the stock concentration of *A. suum* will be determined by incubating eggs at 28°C in an incubator in the dark at the start of the experiment, which is corresponds to Treatment Day 0; the same day that the reactors are prepared and inoculated with the nylon bags. After three weeks of incubation (Treatment Day 21), one-thousand eggs will be counted under the microscope.

Figure 13. Viable *Ascaris* Ova in Larva Stage, Under Standard Microscopy (Source: CDC, 2013a)

The percent viability will be determined by dividing the number of viable eggs by the total counted. Eggs are only considered to be viable if they contain either L_1 or L_2 larva stages and show motility in response to light under the microscope. An example of a viable egg can be seen in Figure 13.

CHAPTER 4: CONCLUSION AND FIELD-BASED RECCOMMENDATIONS FOR ENVIRONMENTAL PUBLIC HEALTH ENGINEERS

4.1 Conclusion

STHs are a major global public health problem and the MDGs cannot be achieved until this problem is addressed. Without providing access to improved sources of sanitation, STHs and other pathogens will continue to contaminate the environment and cause infection in humans. Composting latrines are considered to be a solution for preventing the spread of STHs, and also incorporate the concepts of resource recovery and beneficial reuse. Unfortunately, the majority are not working as originally designed and do not undergo aerobic decomposition to inactivate *Ascaris* and other STHs. It is important to ensure pathogen destruction in these latrines in order to produce a safe product for handling and for use in agriculture.

A. lumbricoides is very persistent and is not easily inactivated. The ova are used as a hygienic indicator because they have thick shells and are highly resistant to environmental stressors. Therefore, other techniques to destroy pathogens such as increasing storage time or using alkalization/dehydration normally do not work on *Ascaris.* The original goal of composting latrines is to produce a safe product by undergoing aerobic decomposition, and this route should be promoted, if possible as it produces a safe product free of pathogens. The application of urea to inactivate *Ascaris* has its possibilities and should also be further studied. Additional research is needed to ensure that both of these strategies work in the field. The newer prototypes of solar

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latrines, such as those that are used in El Salvador, should seriously be considered over the construction of new DVUD composting latrines, as solar latrines may prove to be more effective in producing a safer product.

The literature review and design of the experimental methods revealed gaps in the current literature. The following areas need further research:

- Surveillance and mapping data on prevalence and intensity of soil-transmitted helminths infections is quite lacking and rudimentary. The global burden of disease due to STHs is enormous, and an increased understanding of country and regional prevalence and worm intensities can help better guide control and planning efforts by governments and organizations.

- In the case of WSPs, removal of pathogens and helminths in wastewater by sedimentation is well recorded, including the quality of the influent and effluent. However, the literature is lacking in studies that examine the characteristics of the sludge, its accumulation patterns, concentration of helminthes in sludge, and the viability of pathogens located in the sludge. In addition, many studies discuss the need to better incorporate the sludge management aspect into the original design of the system by developing sludge management plans from the beginning. These management plans should include a safety plan in order to reduce risk of infection when desludging the WSPs and applying the sludge to land and crops.

- Additional studies that focus on the inactivation of *Ascaris,* by urea and solar heat, in composting or solar latrines in the field should be implemented. There are many studies carried out in the lab, and these studies tend to include higher moisture contents than what one would encounter in dry sanitation. Few studies, whether in the lab using parameters that simulate dry latrines, or

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in composting or solar latrines in the field, have been conducted. The lab experiments found in the literature appear to be promising in terms of inactivating *Ascaris*; however, this needs to translate over into the technologies in the field.

4.2 Recommendations

Recommendations for moving forward with research and programming in the field related to increasing access to improved sanitation through dry sanitation methods include:

- Conducting this proposed experiment in the Bocas del Toro region of Panama and repeating it in other parts of the country and Central America.

- Carrying out the proposed experiment, but rather than creating reactors, mix urea directly into the latrine contents, and add the nylon bags to the biosolids in the chamber. Test how many days it takes for inactivation while recording temperature, humidity, pH, ammonia gas levels, etc.

- Taking this one step further by mixing urea directly into the latrine contents within the chamber and taking grab samples of the biosolids in order to determine if *Ascaris lumbricoides*, present in the latrines, becomes inactivated over time. Measure parameters such as temperature, humidity, pH, ammonia gas levels, etc. as explained in the proposed experimental design for this manuscript.

- Repeat all of these experiments in solar latrines in order to compare the results with those of composting latrines to determine which form of dry sanitation is most effective.

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