GLOBAL WATER PATHOGEN PROJECT

PART THREE. SPECIFIC EXCRETED PATHOGENS: ENVIRONMENTAL AND EPIDEMIOLOGY ASPECTS

ASCARIS SPP.

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Summary

Ascariasis is a disease caused by infection with the intestinal roundworm, *Ascaris lumbricoides*. *A. lumbricoides* is regarded as a different species from the pig ascarid, *A. suum*, which has also been reported infective to humans. *A. lumbricoides* occurs worldwide, but it is more prevalent in tropical and subtropical countries, in humid and warm climates, and areas where poverty, poor hygiene, and inadequate sanitation prevail. Over eight hundred million people are infected globally, with the greatest burden in young children.

Adult worms are creamy white to yellowish, often translucent with a pinkish tint. Females are 200 to 490 mm long and 3-6 mm wide. Males are 150-310 mm long and 2-4 mm wide. Eggs appear golden brown, round to ovoid, measuring 45-75 by 35-40 µm.

People become infected with *Ascaris* by ingesting the infective eggs, possibly as contaminants of food or drink. In the intestine, the eggs hatch into larvae, which migrate through the liver to the lungs, up the trachea and are then swallowed, thereby returning to the intestine where they mature as adult male and female worms. Following copulation, females start releasing eggs about seventy days after ingestion of eggs. Diagnosis is by detection of eggs in host faeces. *Ascaris* eggs must embryonate in the environment for around 3-6 weeks, depending on temperature, before they become infective, and can persist in the environment for over six years.

Most infections are asymptomatic but some may show signs typical of asthma or pneumonia in the early stages. Abdominal pain, nausea, vomiting, malnutrition, passing of worms with vomit or in stool, bowel obstruction and pancreatitis may occur in later stages.

In endemic areas, the immediate environment is contaminated due to open defecation, and people may have life-long exposure to infection. Control involves environmental sanitation, chemotherapy, health education, and prevention of indiscriminate defecation, improper disposal of human excreta and use of untreated night soil for manure in agriculture.

As efficacious drugs are available, regular mass drug administration, targeted at school-age children, is currently being adopted by the WHO for the integrated control of ascariasis globally.
However, the impact of this intervention will depend on the provision of safe water supply and the facility for safe disposal of human excreta. Without this infrastructure, the environment will remain a source of infection due to contamination.

1.0 Epidemiology

Human and animal ascariasis together constitutes one of the most important global public health challenges. The disease is widespread and is found worldwide but particularly more common in Sub-Saharan Africa and Southeast Asia (Bethony et al., 2006; Pullan et al., 2014). Transmission is through the faecal-oral route; eggs are ingested following contact with fecally or sludge contaminated food and/or soil, or the deliberate act of eating contaminated soil (Bethony et al., 2006). Thus, ascariasis is a perfect example of an environmental disease.

1.1 Global Burden of Disease

1.1.1.Global distribution

Ascaris lumbricoides is cosmopolitan in distribution. However, *A. lumbricoides*, like the other soil-transmitted helminths (STHs), *Trichuris trichiura* and the hookworms, *Ancylostoma duodenale* and *Necator americanus* thrive in places where the soil is warm and humid, sanitation is poor and poverty and ignorance prevail (Asaolu et al., 2002a). It is estimated that in 2010 about 818 million (771.7-891.6 million) people were infected with *Ascaris lumbricoides* (Pullan et al., 2014). Ascariasis is prevalent in at least 150 of the 218 countries of the world. The overall global prevalence was roughly estimated at 22% (Crompton 1989). The distribution of the global burden of ascariasis shows that South America, Central America and the Caribbean host 8.3%, Africa and the Middle East, 16.7% as well as Central and Southeast Asia and the Oceania region 75% of cases (Shoff, 2015).

In many endemic communities, prevalence may be in excess of 80%. Prevalence varies widely among the endemic countries and the worm presents a patchy and uneven distribution among the communities in the endemic countries, and among different households in the same community (Robertson et al., 1989). In Nigeria, for example, surveys from various authors showed that prevalence varied from 0.9 to 98.2% in places (Crompton, 1989; Holland and Asaolu, 1990). People are exposed to infection from birth and are continually at risk from re-infection because of poor sanitation, low standard of personal hygiene and constant exposure to enormous numbers of *A. lumbricoides* eggs discharged by the female worms to the environment through the faeces of infected subjects daily.

All ages are susceptible to ascariasis although prevalence and intensity are usually higher among school-age children than other age groups. Classical age related distribution patterns indicate that prevalence peaks between ages 4 and 14 years. Intensity of infection measured by the number of worms expelled by the host after chemotherapy or the number of eggs counted per gram of host faeces shows that children harbour more worms than adults. The higher rates observed among the children appear to be related to higher exposure levels among children than immunity to infection among older age groups. Distribution of *A. lumbricoides* as in other parasitic worms, in a population, is known to be over-dispersed, such that majority of individuals in the population carry few or no worms while a smaller percentage of people are heavily infected (Anderson and May, 1991).

Since 2001, the target was set by the World Health

Organization (WHO) for endemic countries to ensure that at least 75% of school-aged children at risk of soil-transmitted helminthiases would have access to treatment with anthelmintic drugs by 2010. The adoption of preventive chemotherapy by the mass distribution of anthelmintic to school-age children by the WHO for the global control of the soil transmitted helminths (STHs) (WHO, 2006a) has since led to considerable reductions in the prevalence and intensity of *A. lumbricoides* and the other soil-transmitted helminths in many of the endemic countries (Uniting to Combat NTDs, 2015).

1.1.2 Symptomatology

A. lumbricoides infections show no symptoms in more than 85% of infected people, especially when the number of worms present is small (Dold and Holland, 2011). Moderate to heavy infections cause various symptoms, depending on which part of the body is affected. Several larvae migrating from the intestine to the other organs are destroyed in the liver and lungs. The remains of the disintegrating larvae induce eosinophilic reactions. In the lungs, the migrating larvae cause slight cough, fever and skin rashes for a few days or, in areas where transmission is seasonal, there are severe symptoms of pneumonitis similar to asthma, persistent cough, shortness of breath, substernal pain and wheezing (Coles, 1985). Adult worms residing in the intestines may induce vague abdominal pain, nausea, vomiting, diarrhoea or bloody stools, nutrient malabsorption and increased nutrient losses in mild infections. In heavy infections, and especially in children, there may be restlessness, loss of appetite, severe abdominal pain, intermittent loose stools, constipation, fatigue, vomiting, weight loss, malnutrition, passing of worms from the anus or the mouth, abdominal distension and abnormal abdominal sounds.

1.1.2.1 Morbidity

The pathological problems associated with *A. lumbricoides* infection can be examined in four specific stages (a) larval migration, (b) adults worms in the small intestine, (c) complications and (d) allergic reactions (Pawlowski and Arfaa, 1985).

The adult worms in the intestine cause disordered changes in the mucosa and the muscle layers. There is villus atrophy, coarsening of mucosal folds, shortening of crypt depths, reduced mucus production and hypertrophy of the muscle layers of the intestinal wall (Tripathy et al., 1972). Chronic heavy infection of school-aged children in developing countries significantly contribute to protein energy malnutrition, reduced food intake, retarded growth, cognitive deficits and mental retardation (Stephenson et al., 1983).

The migrating larvae provoke inflammatory reactions in the intestinal mucosa, liver and lungs. Some of them become trapped and immobilised by eosinophils, leading to granuloma formation. In the lungs, the larvae moving from the blood capillaries into the air spaces puncture the capillaries resulting in haemorrhage and oedema of the alveoli. Alveolar sacs become filled with serous exudate. There is infiltration of the peri-bronchial tissues with eosinophils and neutrophils with increased mucous production in the bronchi. This gives rise to cough, high fever, and bronchial asthma (Asaolu et al., 2002b). This condition, regarded as Loeffler effect, results in transient lung infiltration with blood eosinophilia, and is frequently caused by *Ascaris* larvae migrating through the lungs. The reaction is severe when the number of larvae is large or when transmission is seasonal as recorded by Gelpi and Mustafa (1967) in Saudi Arabia.

Severe complications in Ascaris infection are frequently associated with the large size and aggregating and migratory habits of the worm. Migration of worms from their normal location in the intestine can be induced by fever, ingestion of some drugs or food, and administration of surgical anaesthesia in the host. Worms which move to the stomach may become vomited (Villamizar et al., 1996). Obstruction of the upper respiratory tract has also been reported (Andrade-junior et al., 1992; Astral and Rogers, 1995; Pawlowski and Arfaa, 1985; da Silva et al, 1997; Villamizar et al., 1996; Xianmin et al., 1999). In heavy infections several worms may ball up to form a bolus and cause intestinal obstruction which may be accompanied by complications such as intussusceptions, volvulus, haemorrhagic infarction and perforation of the wall of the intestine (Tripathy et al., 1971; Blumenthal and Schultz, 1975; Crompton, 1989). Worms can invade the bile ducts, the liver, pancreatic duct, the appendix, surgical wounds and the peritoneum (Wani et al., 2010) where they cause abscesses.

A fatal case of multiple liver abscesses by *A. lumbricoides* in a 21-month-old child was reported in Sao Paulo by Rossi and Bisson (1983). In a fatal infection of a 2-year-old South African girl, 796 *Ascaris* were recovered at autopsy from the ileum which had suffered torsion and gangrene (Baird et al., 1986). Nonfatal heavy infection cases of up to 990 worms have been recorded while there have been infections of intestinal obstruction with only 4 worms. The average worm burden in fatal cases was 659 (23-1978). Even infection with a single adult worm can be fatal if it migrates into the common bile duct, the pancreatic duct or the appendix. The frequency of such complications may be very low but they represent the main causes of acute abdominal emergencies in children in many endemic countries. The disability adjusted life years lost for ascariasis is estimated as 10.5 million years.

According to Coles (1985), the main cause for allergy and immunopathology to *Ascaris* appears to be raised anti-*Ascaris* IgE reacting with allergen and causing release of histamine. Laboratory workers who are not infected with *Ascaris* but who have inhaled antigens from the eggs, larvae or adults of the worm, have experienced allergic reactions such as asthma, eosinophilia, gastrointestinal disorder and urticarial (Arfaa, 1984; Tripathy et al, 1971). Presumably these workers are sensitized by inhaling and thereby swallowing airborne antigens escaping from the worm materials they handled in the laboratory. Hence it has been suggested that *Ascaris* is the most potent and therefore dangerous source of allergens routinely handled in the laboratory. It has been suggested that the abdominal

symptoms of ascariasis are allergic in nature and must arise from the production of a toxin, or a peptide causing release of histamine, or allergens that result in immunopathology or a combination of these factors (Coles, 1975, 1985).

Annual global mortality due to ascariasis was initially estimated to range from 10,000 to 200,000 but more realistically, it was believed to be about 10,000 deaths per year (da Silva et al., 1997). The death rate may have been reduced considerably by the ongoing global integrated control programme of soil-transmitted helminths (of which *A. lumbricoides* is among) by the WHO (Uniting to Combat NTDs, 2014). In 2013, the global death rate ascribed to ascariasis was estimated at 4,500 (Hotez and Herricks, 2015), but the data are not available.

1.2 Taxonomic Classification of the Agent

1.2.1 Taxonomy and physical description of the agent

Ascaris lumbricoides is a parasitic worm which causes ascariasis disease in humans. It is the most common and prevalent nematode parasite infecting humans (Croll et al, 1982). Up to seventeen species of Ascaris have been recognized inhabiting the gastrointestinal tracts of mammalian hosts (Crompton, 1989; Shoff, 2015). A. lumbricoides has high host specificity for humans but it can also infect and reach sexual maturity in pigs. The worm has been reported to develop to some extent in other animals like bears and primates but in these hosts, the parasite has not been demonstrated to become sexually mature and produce eggs. Ascaris suum, a closely related species, has high host specificity for pigs but patent infections have also been reported in humans (Galvin, 1968; Eddy, 1985). A. suum has also occasionally been reported in cattle and sheep but has not attained sexual maturity in them. Questions have been raised as to whether A. lumbricoides and A. suum are the same or separate species in view of the fact that the two nematodes are indistinguishable morphologically, and they and their human and pig hosts often co-exist. However, detailed studies on the morphology, protein profiles, sex chromosomes and molecular genetics of the two worms have revealed important differences which imply that the two nematodes are separate species (see Crompton, 1989).

Ascaris lumbricoides is the type species of the genus and its systematic position is presented in Table 1.

Table 1: Classification of *Ascaris lumbricoides* (according to Guy, 2017)

Kingdom	Animalia
Phylum	Nematoda
Class	Secernentea
Order	Ascaridida
Family	Ascarididae
Genus	*Ascaris*
Species	*A. lumbricoides*

1.2.2 Morphology

Ascaris lumbricoides is the largest nematode inhabiting the human alimentary tract, usually found residing in the jejunum of the small intestine (Arfaa, 1984; Crompton and Pawlowski, 1985; Andrade-junior et al., 1992). Adult life worms are turgid and the colour is creamy white with pinkish tint. Male worms measure 15 to 31 cm in length, 2 to 4 mm in width and have posterior end curved ventrally. Female worms are larger, 20 to 49 cm in length and 3 to 6 mm in width. Eggs released by the females are passed with host faeces. Both fertilized and unfertilized eggs are usually detected in faeces and both appear brown in colour. Fertilized eggs are round to oval, 45 to 75 by 35 to 50µm in size, containing a developing embryo. The egg is covered by a thick shell made of four layers. The outermost layer appears rough or irregular on the surface and consists of sticky mucopolysaccharide, contributed from the uterine wall of the parent female worm (Foor, 1967). This layer may be removed from many fertilized eggs, giving them a more or less smooth surface. The otherthree layers are secreted by the embryo; they are composed of an outer thin proteinaceous membrane, a middle protein and chitin layer that provides structural strength, and the innermost ascarocide layer, which consists of protein (25%) and unsaponifiable lipid (75%). The ascarocide layer is selectively permeable and is important for the survival of the eggs in various hazardous conditions (Wharton, 1980; Perry and Clark, 1981). The unfertilized eggs are larger and elongated in shape, measuring 60 to 100 by 40 to 60µm.

1.3 Transmission

1.3.1 Life cycle

A detailed life cycle of *A. lumbricoides* has been described by Crompton and Pawlowski (1985) and more recently by the United States Centre for Disease Control (Figure 1) (CDC, 2015). At a temperature of 25-30°C, high humidity and adequate oxygen supply, fertilized eggs released in the environment moult once and embryonate in 15-35 days to become infective.

The infective egg contains a second stage larva which measures 50-70 x 40-50 µm within the egg shell. Infection occurs by ingesting infective eggs with contaminated food materials and/ or soil. The eggs hatch in the jejunum into larvae within a few hours of being swallowed. The larvae penetrate the intestinal mucosa and migrate via the portal vessels and the lymphatic system into the liver in 2-8 days. The larvae at this stage, in experimentally infected rats, measure 258 x 14 µm. From there they are carried through the heart to the lungs. They penetrate the capillary walls and enter the lung alveoli where they measure 564 x 28µm.

They spend about ten days in the lungs when they moult twice to the fourth stage larvae and grow to a size of 1700-2000 µm. Then they are coughed up the bronchi and trachea to the pharynx from where they are swallowed. The larvae pass down the oesophagus and through the stomach to the small intestine. On arrival in the small intestine they carry out the fourth and final moult and then form immature adults. The worms mature to adult males and females in 14-20 days and copulation takes place between male and female worms (Shoff, 2015). Fertilized female worms start releasing eggs about 70 days after swallowing infective eggs. A fertilized female worm releases about 200,000 eggs per day and adult worms survive for one to two years in the human host. Majority (about 87%) of the adult worms reside in the jejunum. The eggs released in the intestine are discharged with the host faeces. The fertilized eggs are very resistant to various harsh environmental conditions, are persistent in the environment and can remain viable in the soil for up to six years (Asaolu et al., 2002a). Due to the microscopic size of the eggs, they can be carried in the wind in dry dust from where they can settle on and contaminate various objects in the human environment.

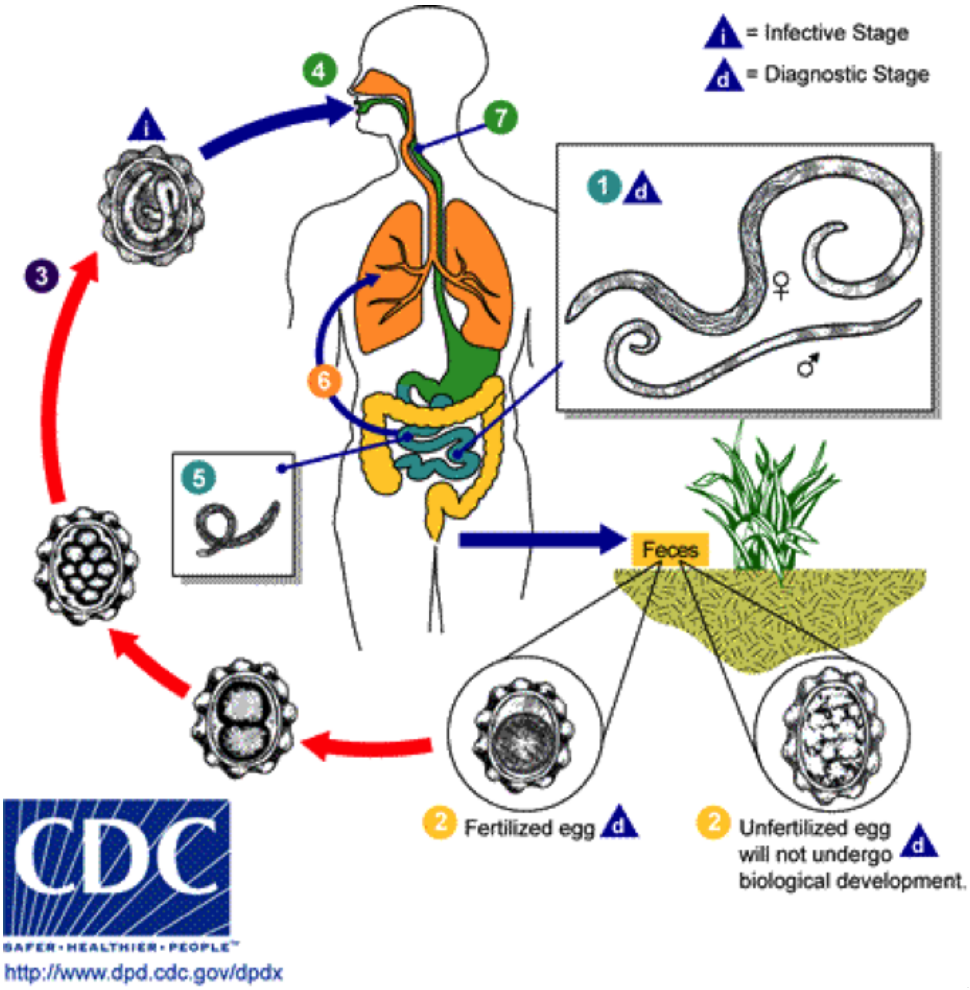

Figure 1. Life Cycle of *Ascaris lumbricoides* (Adult worms [1]. live in the lumen of the small intestine. A female may produce approximately 200,000 eggs per day, which are passed with the feces [2]. Unfertilized eggs may be ingested but are not infective. Fertile eggs embryonate and become infective after 18 days to several weeks [3], depending on the environmental conditions (optimum: moist, warm, shaded soil). After infective eggs are swallowed [4], the larvae hatch [5], invade the intestinal mucosa, and are carried via the portal, then systemic circulation to the lungs [6]. 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 [7]. Upon reaching the small intestine, they develop into adult worms [1]. 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. (Source: Centers for Disease Control and Prevention (CDC, 2015))

1.3.2 Routes of transmission

Transmission of *Ascaris lumbricoides* is through the faecal-oral route, by the ingestion of the infective eggs from soiled hands and food contaminated with human faeces or unprocessed sludge or biosolids taken from sewage treatment systems where the ova accumulate. Both fertilized and unfertilized eggs are released with faeces. Unfertilized eggs do not develop further and are not infective. To become infective, the fertilized eggs must first develop and embryonate outside the host body in soil (or sludge) in warm and moist conditions to the first stage larva and moult once to form a second stage larva within the egg shell. Eggs embryonate in 15-35 days to become

infective in the environment.

In highly endemic areas, with poor hygiene facility and behaviour, and where the human environment is contaminated with human wastes, *Ascaris* eggs can be spread from where they are deposited on the soil on the body of cockroaches, houseflies and other corprophagous insects, or by the wind in dry dust. *Ascaris* eggs have been found to contaminate various domestic objects and public sites such as coins, paper money, door handles, chopping board, fingers, fruits, vegetables, children's play pens and household furniture. The practice of using night soil (human faeces) as fertilizer or untreated wastewater in irrigation also leads to heavy contamination of the soil and vegetables with *Ascaris* eggs and infective stages of other gastrointestinal pathogens. In a study conducted by Amahmid et al. (1999) in Marrakech, Morocco, it was shown that crops irrigated with raw wastewater contained *Ascaris* eggs in the following concentrations per kg of fresh potatoes (0.18), turnip (0.27), coriander (2.7), mint (4.63), carrots (0.7) and raddish (1.64) while crops irrigated with treated wastewater and freshwater were free from contamination. Section 3 of this write-up gives a detailed account of treatments required to inactivate *Ascaris* ova in different media. Hence geophagy (ingestion of soil) and poor food and hand hygiene expose people to ingestion of infective *Ascaris* eggs. Ignorance about the source of infection, lack of potable water supply and inadequate or lack of facility for proper human waste disposal are the major factors contributing to the spread and sustenance of transmission of the worm in most endemic areas.

1.3.3 Intermediate hosts and reservoirs

Ascaris lumbricoides has a direct life cycle and thus has no intermediate hosts. The worm is highly host specific and humans are the major reservoir of infection for the worm.

1.3.4 Incubation period

The prepatent period, that is, time between the ingestion of the worm's eggs to detection of eggs in host faeces, is 67-76 days. A fertilized female worm produces about 200,000 eggs per day (although the number fluctuates) during a life span of 1-2 years.

1.4 Population and Individual Control Measures

1.4.1 Medical treatment options

The medications recommended for treatment by the WHO are albendazole, mebendazole, levamisole and pyrantel pamoate (WHO, 2006a, 2011a, 2011b). Other effective drugs include piperazine and nitazoxanide. Albendazole, also a benzimidazole carbamate, is supplied in tablets of 200 mg albendazole and as suspension of 20 mg albendazole/ml. It is administered as a single dose of 400 mg (2 tablets or 20 ml suspension) to both adults and children above the age of 2 years. Mebendazole, a benzimidazole derivative, kills the worms in the intestine leading to their expulsion within 24 hours of drug administration. Mebendazole is available in oral tablets, each containing 100 mg mebendazole and in suspension containing 20mg mebendazole/ml. Mebendazole and albendazole should not be given during pregnancy because of possible teratogenic effects.

Materials from local herbs, as plant-based anthelminthic, have also been employed in some countries as alternative treatment of ascariasis. The plants from which the active antihelminthic agents are derived include *Prunus mune*, *Asarum heterotropoides*, *Zingiber officinale*, *Coptic chinensis*, *Angelica sinensis*, *Phellodendron amurense*, *Cinnamomum cassia*, *Panax qinseng* and *Zanthoxylum bongeanum* (Li et al., 1991; Lu, 1992; Xianmin et al., 1999; Yi, 1984). In Madagascar, four local herbs found to be effective in the treatment of ascariasis are *Physcalis pemviana*, *Melia azedaracia*, *Chenopodium ambrosiodes* and *Psiadia salviaefolia* (Kightlinger et al., 1996). Epazote, an annual plant called *Chenopodium ambrosioides*, is used as herbal remedy for intestinal worms including ascariasis in South and Central America (Taylor, 2005). Epazote can cause vomiting, dizziness, weakness and convulsions and respiratory and cardiac problems and can be fatal. It is contraindicated in pregnant women because of its toxicity and possible damage it could do to the unborn baby.

Chemoprophylactic measures, by the use of drugs of proven efficacy and safety have been found to be more rapid and more effective in reducing morbidity. In 2001, the World Health Organization endorsed preventive chemotherapy (PCT) as the global strategy to control soil-transmitted helminths (STHs) (WHO, 2006a). The key component of this strategy is regular administration of anthelminthic drugs to at risk groups such as school-aged children, women of reproductive age, and adults in high risk occupations like night soil reuse, farming and others which expose people frequently to contact with soil. The use of chemotherapy for the control of STHs is approached in three ways; mass chemotherapy, group-targeted chemotherapy and treatment of individual cases. The type of chemotherapy control assigned to a community is decided after a survey of the geographical distribution of the prevalence and intensity of infection in that community.

Mass chemotherapy involves treatment of every eligible and willing members of a population at risk without a laboratory diagnosis. Mass treatment has been adjudged to be more economical than the other approaches in that the cost of technical skill and equipment for diagnostic procedure is saved. Mass chemotherapy has been reported as the most effective and has been recommended where more than 20% of the population is affected, treating everyone at regular interval due to reoccurring infections (Albonico et al., 1995; Hlaing et al.1987; Hagel and Guisti, 2010; Dold and Holland, 2011; Jia et al. 2012).

Group-targeted chemotherapy is directed at specific groups of the population who carry the greatest burden of infection, are at higher risk of morbidity and also the greatest source of contamination of the environment with infective eggs. For STHs, the targeted group usually comprises the school-aged children, except in areas of extreme endemicity where all ages are uniformly infected

(Albonico et al., 1996, WHO, 1987). It has been established that in targeted chemotherapy, significant reductions in prevalence and worm burden are achieved not only in the targeted group but also in the untreated segment of the population. This is attributable to the overall reduction in contamination of the environment with infective stages of the parasites with a resultant decrease in exposure of the whole community to infection (Bundy et al., 1990; Asaolu et al., 1991; Albonico et al, 1996).

In situations where there is no organized control programme, individuals may take anthelminthic drugs periodically as a prophylactic measure. Individuals who present with symptoms of infection can be diagnosed at the hospital, where the facility is available, and treated. This approach is useful in identifying and treating individuals carrying heavy worm burden or suffering from a severe manifestation. Hence hospital based individual treatments have been suggested as a possible approach to reducing morbidity due to ascariasis in a community (Asaolu et al., 1991).

1.4.2 Vaccines

Humans make antibodies (predominantly IgE) in response to *Ascaris* antigens and infection and the response is believed to confer some immunity. Although several efforts have been made to develop vaccines for effective protection against ascariasis infection, none has proved effective (Hagel and Guisti, 2010). Future research may lead to the development of vaccines that will permit better control and management of the parasite.

1.4.3 Hygiene measures

It has been emphasized that preventive chemotherapy (WHO, 2006a) should be applied together with hygiene measures, without which reinfection is inevitable. Hygiene measure against *A. lumbricoides* is through improved access to sanitation. This includes safe disposal of human excreta (Ziegelbauer et al., 2012) by the use of properly functioning toilets or latrines by all community members (Dold and Holland, 2011), and when wastewater is produced, its proper treatment and disposal especially the sludge. It also requires provision of safe and adequate water supply for personal and domestic hygiene (Asaolu and Ofoezie, 2003). Personal hygiene includes washing of the hands before eating and after visiting the toilet. Domestic hygiene requires water to keep food, utensils and home clean (Fung and Cairncross, 2009). Studies in some communities in Iran (Zarga and Khuroo, 1990) have shown positive impacts of the combination of water supplies and excretal disposal on the prevalence and intensity of infection of ascariasis. In St. Lucia, West Indies, prevalence of *A. lumbricoides* fell by 31% among a group of children with household water and latrine compared with a control group without such facilities (Henry, 1981).

2.0 Environmental Occurrence and Persistence

2.1 Detection Methods

According to Collender et al. (2015), quantifying environmental contamination with *Ascaris* eggs poses major technical challenges to effective monitoring of control programmes. Methods are, therefore, needed that are efficient and sensitive enough to detect low but epidemiologically relevant concentrations in different environmental media and cost effective enough to be deployed in low resource settings where the impact of ascariasis is high (Collender et al., 2015). A systematic review of the literature shows that there are several methods available for assessing the occurrence and intensity of *Ascaris* ova in different environmental media such as water including surface water, drinking water and seawater; soil including night soil, sewage, sludge, biosolids and faecal materials.

The commonest of the methods are based on flotation, sedimentation, concentration and microscopic examinations. Table 2 presents the list of the most commonly used methods, their sensitivities and power of prevalence estimate of *Ascaris* ova in faeces. Also presented in the table are the sensitivity and prevalence estimates of different combinations of the methods. Unfortunately, information on the specificity and predictive values of the methods are either too scanty or non-existing. According to Goodman et al. (2007) whom presented the most comprehensive data on sensitivity, the most sensitive single method was sedimentation (91.7%) while sedimentation and Kato Katz gave the most sensitive combination (100%).

Table 2: Prevalence estimates of comparing different methods used to quantify *Ascaris lumbricoides* eggs in stool

Method	Prevalence[a] % (Range)	Sensitivity[b] (%)
Kato Katz (KK)[c]	10.2 (7.1 to 13.3)	77.1
Modified Wisconsin (MW)	9.1 (6.1 to 12.1)	68.8
Sedimentation (S)	12.1 (8.8 to 15.5)	91.7
Formol ethyl acetate (FEA)[c]	8.3[c] (5.4 to 11.1)	62.5[c]
Modified formol ethyl acetate (MFEA)	9.1 (6.1 to 12.1)	68.8
KK + MW	10.5 (7.3 to 13.6)	79.2
KK+S	13.2 (9.7 to 16.7)	100
KK + FEA	11.0 (7.8 to 14.3)	83.3
KK + MFEA	10.7 (7.5 to 13.9)	81.1
MW+S	12.7 (9.2 to 16.1)	95.8
MW + FEA	10.2 (7.1 to 13.3)	77.1
MW + MFEA	9.9 (6.8 to 13.0)	75.0
S + FEA	12.1 (8.8 to 15.5)	91.7
S + MFEA	12.4 (9.0 to 15.8)	93.8
FEA + MFEA	10.2 (7.1 to 13.3)	77.1

[a]Percentage of ova found in feces by a comparative study (Goodman et al., 2007); [b]Probability of accurate positive diagnosis (Goodman et al., 2007); [c]Periago et al. (2015) study comparing Kato Katz (KK) and Formol ethyl acetate (FEA) and found an equivalent prevalence of 20 and 19.4 and a sensitivity of 97.2 and 94.2, respectively; [d]Nikolay et al. (2014) compared sensitivities of: Direct microscopy, Kato Katz (KK), FLOTAC, Mini FLOTAC, Formol ethyl acetate (FEA), and McMaster and found 52.1, range of 63.8 to 70.4, 79.7, 75.5, 56.9, and 61.1, respectively

Table 2b. Sensitivity of different methods used to quantify *Ascaris lumbricoides* eggs in stool

Method	Prevalence (%)(Range)		Sensitivity[a](%)		
	Goodman et al., 2007	Periago et al., 2015	Goodman et al., 2007	Periago et al., 2015	Nikolay et al., 2014
Direct microscopy	NR[b]	NR	NR	NR	52.1
Kato Katz (KK)	10.2 (7.1 to 13.3)	20	77.1	97.3	63.8 to 70.4
Modified Wisconsin (MW)	9.1 (6.1 to 12.1)	NR	68.8	NR	NR

Method	Prevalence (%)(Range)		Sensitivity[a](%)		
	Goodman et al., 2007	Periago et al., 2015	Goodman et al., 2007	Periago et al., 2015	Nikolay et al., 2014
Sedimentation (S)	12.1 (8.8 to 15.5)	NR	91.7	NR	NR
FLOTAC	NR	NR	NR	NR	79.7
Mini FLOTAC	NR	NR	NR	NR	75.5
Formol ethyl acetate (FEA)	8.3 (5.4 to 11.1)	19.4	62.5	94.2	56.9
Modified formol ethyl acetate (MFEA)	9.1 (6.1 to 12.1)	NR	68.8	NR	NR
KK + MW	10.5 (7.3 to 13.6)	NR	79.2	NR	NR
KK+S	13.2 (9.7 to 16.7)	NR	100	NR	NR
KK + FEA	11 (7.8 to 14.3)	NR	83.3	NR	NR
KK + MFEA	10.7 (7.5 to 13.9)	NR	81.1	NR	NR
MW+S	12.7 (9.2 to 16.1)	NR	95.8	NR	NR
MW+FEA	10.2 (7.1to 13.3)	NR	77.1	NR	NR
MW + MFEA	9.9 (6.8 to13.0)	NR	75	NR	NR
S + FEA	12.1 (8.8 to 15.5)	NR	91.7	NR	NR
S + MFEA	12.4 (9.0 to 15.8)	NR	93.8	NR	NR
FEA + MFEA	10.2 (7.1 to 13.3)	NR	77.1	NR	NR
McMaster	NR	NR	NR	NR	61.1

[a]Probability of accurate positive diagnosis; [b]NR: Not Reported

However, though data presented by other workers (Nikolay et al., 2014; Periago et al., 2015) were comparatively scanty, it is obvious that both prevalence estimates and sensitivity of each method varied substantially from one investigation to another. Also sensitivity appeared to increase with increasing prevalence estimate, although data presented may not sufficiently support this conclusion. Table 3 gives the outcome of McMaster method using different protocols to assess capacity to recover *Ascaris* eggs in a given medium. The lesson here is that outcome of even the best method may depend on the actual protocol selected. Using the mean recovery rate, Jeandron et al. (2014) showed that McMaster was over 33% better than FLOTAC. the performance of McMaster varied substantially depending on the type of tubes, pipettes and detergents used. Its worst performance (33.4% recovery rate) was recorded with the use of Falcon tubes and coated glass pipettes. Conversely, it performed best (89.4% mean recovery rate) when detergent 7X 1% was used. Besides the egg recovery efficiency, the challenge remains notably in environmental samples that are not too clean, for instance sludge, biosolids and wastewater. In such media, proper identification of eggs in a mixture of particles is very difficult under a microscope (Maya et al., 2006).

Table 3: Mean recovery rate of *Asaris* ova on hand by different methods (Source: Jeandron et al., 2014)

Method	Mean egg recovery rate (%) (95% CL) (n=5)
FLOTAC (basic method)	43.3 (26.4 to 60.1)
McMaster	64.8 (52.6 to 77.1)
McMaster with:	
Falcon tubes NC and glass pipettes NC	58.4 (33.2 to 83.6)
Falcon tubes NC and glass pipettes CC	57.0 (30.7 to 83.2)
Falcon tubes C and glass pipettes C	33.4 (0 to 74.0)
Falcon tubes C and plastic pipettes NC	37.3 (0 to 86.5)
Falcon tubes C and glass pipettes NC	35.5 (0 to 95.4)
McMaster and various detergents viz: 7X 1%	89.4 (67.1 to 100)
Tween 80 0.1%	58.5 (32.5 to 85.5)
Benzethonium chloride 0.1%	86.7 (73.7 to 99.8)
Cetylpyridinium chloride 0.1%	84.2 (65.7 to 100)
Deionized water (control)	74.0 (67.3 to 80.7)

2.1.1 Methods used to assess viability of *Ascaris* ova in different environmental media

The increasing use of sewage sludge and biosolids in agriculture has increased the number of *Ascaris* ova in the environment (Simonart et al., 2003). Besides, the resilience and hardiness of *Ascaris* ova exacerbate rather than mitigate the problem. Subsequently, ascariasis is now a major risk to public health especially in the tropical areas where poverty and environmental conditions that favour its transmission also exist. As already stated, breaking transmission requires that contaminated environmental media are identified and eggs inactivated as will be described in the next section. However, presence of *Ascaris* ova is not always an indication of potential transmission unless the eggs are confirmed to be viable. This is because non-viable eggs cannot transmit disease and treating non-viable eggs is wasteful in terms of resources and efforts. It is therefore important to identify methods that can determine not only the presence of *Ascaris* eggs in a medium but also discriminate between viable and nonviable status. The importance of this is not farfetched. Presence of viable eggs in a sample is a clear indication of risk to public and/or livestock health and the use of biosolids and compost manure in agriculture increases this risk.

There are currently several methods available for the assessment and enumeration of viable *Ascaris* eggs in several media (Table 4). These methods can broadly be divided into two major groups; (i) methods based on optical microscopy and (ii) methods based on molecular technologies. The microscopy based methods are further divided into those that require the use of vital dyes and those that do not require staining. The first group include the Tulane method developed by Reimers et al. (1981) and its modified version the Yanko method. Others are the Gaspard, the US EPA and the Victorian methods. The Triple flotation and the Norwegian methods were modifications of the US EPA method that incorporated staining technology. The principle of staining is based on the fact that usually live and dead eggs react differently to vital dyes and give out different colours. The cell membrane of live cells usually absorbs dyes, while membranes of dead cells are impermeable to dyes. For instance, using the LIVE/DEAD BacLight Bacterial Viability kit, Dabrowska et al. (2014)

reported that viable eggs stained red while dead eggs stained green. The most prominent molecular technique is the quantitative polymerase chain reaction (rPCR) developed by Pecson et al. (2006). This method is based on the theoretical concept that first internally transcribed spacer (ITS-1) region of ribosomal DNA (rDNA) and rRNA levels increase proportionally with the number of cells in an *Ascaris* egg. Thus, quantitative estimate of ITS-1 level is a good index for detecting non-viable, viable and larvated eggs.

The major difference between the microscopy methods is in the solutions used in the different steps of desorption, flotation, extraction and incubation. Another difference is that some perform the extraction step after the incubation of eggs to induce hatching of embryonated eggs, such as the Tulane method and its modifications (Reimers et al., 1981; Gaspard et al., 1996). USEPA method is, however, the most commonly and widely used although there are complaints that it is too tedious, time consuming and involves too many steps, including filtration and incubation that may last for weeks (Simonart et al., 2003). The general consensus is that among the incubation based methods, none combines both speed and sensitivity in the detection of viable *Ascaris* eggs (Vieira da Rocha et al., 2016).

Raynal et al. (2012) reported that the recent application of polymerase chain reaction (PCR) could for the first time provide a procedure that is both fast and reliable. However, Choi et al. (2007) reported that while this may be true, conventional PCR can only identify presence or absence of particular *Ascaris* species in a sample, but cannot quantify their levels. This limitation has now been corrected by the development of incubated quantitative PCR (qPCR) which is capable of quantifying the levels of viable eggs in samples (Pecson et al., 2006; Dabrowska et al., 2014). In spite of this, the prevalence of false positives is still high and efforts are being made to provide an effective tool that is both effective and sensitive (Vieira da Rocha et al., 2016). The Reverse Transcriptase-PCR (RT-PCR) which uses RNA rather than DNA has been suggested as an option against this weakness.

However, Rocha (2015) argued that even when this is true, there are no technologies available for relating the quantity of RNA in a sample to the number of viable *Ascaris* eggs. Thus, while the potential of RT-PCR to reduce false positive in samples is high, its application may have to wait till a procedure is found that relates the quantity of RNA in sample to the number of viable *Ascaris* eggs.

Table 4a: Major methods used for assessing viability of *Ascaris* ova in different environmental media

Method	Surfactant	Floatation liquid	Extraction	Incubation	Reference
Tulane	Limbro 7X	$MgSO_4$	Sieving þ10% NaClO	Formalin 0.5%	Reimers et al., 1981; Bowman et al., 2003
Yanko	Hot tapwater	$ZnSO_4$	Acid alcohol	H_2SO_4 0.1N	Yanko, 1987
Gaspard	0.01% SDS	NaCL	Ultrasonication þ NaClO	Deionized water	Gaspard et al., 1996
USEPA	Limbro 7X	$MgSO_4$	Sieving	H_2SO_4 0.1N	USEPA, 2003
De Victorica and Galvan	NaCl .85%	$ZnSO_4$/ $MgSO_4$	Membrane filter	None	de Victorica and Galvan, 2003; Pecson et al., 2006

Table 4b: Major methods used for enumerating the viability of *Ascaris* ova in different environmental media

Method	Floatation liquid	Microscopy	Viability classification*	Reference
EPA Modified[a,b]	$NaNO_3$	Yes	Embryonated	USEPA, 1999; Simonart et al., 2003
Triple Flotation[a]	$ZnSO_4$	Yes	MTT dye (blue)	Simonart et al., 2003
Norwegian Method[a]	Sucrose	Yes	Embryonated	Simonart et al., 2003
Quantitative Polymerase Chain Reaction (qPCR)	?????	Yes	Stain green	Dabrowska et al., 2014

2.2 Data on Occurrence in the Environment

2.2.1 Occurrence in excreta in the environment

There are many sources of human excreta in the environment. These include but not limited to open defaecation, evacuation of pit latrines, compost latrines and vault latrines (Esrey, 1998). Consequently, Ascaris ova could be more widespread in the environment than imagined (Table 5). The table shows clearly that *Ascaris* ova occurred in all environmental media investigated at varying prevalence. For instance, prevalence of *Ascaris* ova in soil ranged from 8.8% at Umuji in Nigeria (Chukwuma et al., 2009) to 84.4% in Turkey (Ulukanligil et al., 2001). The corresponding prevalence range for other media were for faecal matter 29.6% in Nigeria (Odu et al., 2013) to 88.5% in Turkey (Ulukanligil et al., 2001); water samples 5.0% in Pesing Polgar (Subahar and Sutanto, 2008) to 60.8% in Turkey (Ulukanligil et al., 2001). Other media where *Ascaris* ova were recorded included vegetables (14%), manure 30% (at the middle heap) to 88.0% (at the bottom heap), human nails (65%) and hands (60%).

Table 5. Prevalence of *Ascaris* ova in different media in Nigeria and Turkey

Area	In Soil Percent Positive (# of Samples)	In Faecal Matter Percent Positive (# of Samples)	Reference
Nigeria			
Umuji	8.8 (7/80)	50 (40/80)	Chukwuma et al., 2009
Umuogbuefi	30 (18/60)	50 (40/80)	Chukwuma et al., 2009
Obuno	27.5 (22/80)	65 (39/60)	Chukwuma et al., 2009
Choba	NR[a]	29.6 (8/27)	Odu et al., 2013
Rumuolumeni	NR	70.4 (19/27)	Odu et al., 2013
Nwofe-Agbaje	30 (18/60)	NR	Nwoke et al., 2013
Omu-Ebonyi	33.3 (20/60)	NR	Nwoke et al., 2013
Ntezi-Abba	28.3 (17/60)	NR	Nwoke et al., 2013

Area	In Soil Percent Positive (# of Samples)	In Faecal Matter Percent Positive (# of Samples)	Reference
Umuogharu	36.7 (22/60)	NR	Nwoke et al., 2013
Ezzagu	25 (15/60)	NR	Nwoke et al., 2013
Turkey			
Turkey	84.4 (76/90)	88.5 (69/78)	Ulukanligil et al., 2001

[a]NR: Not Reported

2.2.2 Occurrence in sanitation facilities

Table 6 presents the prevalence of *Ascaris* ova in the major sanitation facilities examined in Nigeria and Kenya. Clearly, *Ascaris* ova occurred in all the facilities recorded in the literature. However, prevalence is higher in the pit latrines and bush defaecation sites than in around water closet. This can be explained partly by the socio-economic divergences of people using each type of latrine and the physico-chemical characteristics of the latrines (Walls, 2015).

Prevalence of viable *Ascaris* eggs in fresh and old samples of faeces and compost can be assessed by different viability test methods. The various methods tried to compare the capacity of the tools to discriminate among dead eggs, viable eggs and embryonated eggs in fresh and old samples (Table 7). The samples investigated included compost, UV inactivated eggs and faecal sludge.

Table 6. Prevalence of *Ascaris* ova in different latrine facilities in Nigeria and Kenya

Area	Water Closet Percent Positive (# of Samples)	Pit Latrine Percent Positive (# of Samples)	Bucket Percent Positive (# of Samples)	Open Defaecation Percent Positive (# of Samples)	References
Kenya					
Kenya	11.7 (17/145)	11.8 (41/347)	0 (0/1)	16.7 (1/6)	Adoko, 1985
Nigeria					
Imo State	6.3 (1/16)	32.3 (70/217)	NR[a]	29.4 (15/51)	Odinaka et al., 2015
Choba	10.1 (16/158)	NR	NR	NR	Odu et al., 2013
Rumuolumeni	18.3 (15/82)	26.7 (16/60)	NR	NR	Odu et al., 2013
Akure	52.4 (22/42)	41.9 (44/105)	53.3 (8/15)	NR	Dada and Aruwa, 2015
Akure	18.8 (6/32)	72.9 (107/148)	NR	72.9 (124/170)	Dada, 2016
Zamfara	1.5 (9/600)	67.8 (407/600)	NR	7.7 (46/600)	Shehu et al., 2013

[a]NR: Not Reported

Table 7. Prevalence of viable *Ascaris* eggs in fresh and old faecal samples assessed by different viability test methods

Area	Matrix	Method	Treatment	Fresh Samples Percent Positive (# of Samples)	10 Day Old Samples Percent Positive (# of Samples)	Reference
Haiti	Compost of faeces	US EPA method[a]	Composting	10.4 (94/900)	0.46 (14/3055)	Berendes et al., 2015
NY, USA	Stock solution	US EPA method[a]	UV	21.8 (44/202)	11 (23/210)	Raynal et al., 2012
NY, USA	Stock solution	US EPA method[a]	High heat (70°C for 30s to 3min)	0.01 (2/275)	0 (0/258)	Raynal et al., 2012
NY, USA	Stock solution	US EPA method[a]	Moderate heat (40°C for 6 to 24h)	0 (0/124)	0 (0/144)	Raynal et al., 2012
NY, USA	Stock solution	US EPA method[a]	Moderate heat (40°C with 2000 mgl-1NH_3-N)	0 (0/96)	0 (0/88)	Raynal et al., 2012

[a]94 viable *Ascaris* ova found in only fresh untreated compost and none in compost older than 6 weeks; 76 embroyanated ova (infectious) were also found in the fresh untreated compost and none in compost older than 2 weeks

All the methods showed that there are more viable eggs in fresh samples than in old samples. For instance, the US EPA showed that composition of viable eggs in fresh samples could range from 10.4% in fresh compost to 21.6% in faecal sludge. In the compost, the method identified 79.8% of the viable eggs as being embryonated. It is also interesting to note that modified US EPA methods identified more eggs as viable compared with the original method, the most sensitive being modified US EPA method with sieving. Each of the methods identified as viable less eggs in older samples. Although not shown in this table, the number of viable eggs identified by the methods decreased with increasing age of samples. After two weeks, no viable eggs were found in any sample (Raynal et al., 2012; Berendes et al., 2015). Factors which affected the rate and length of viability included temperature, moisture and location of sampling (i.e. centre or side vault). While Berendes et al., (2015) reported that Ascaris eggs at the centre die-off faster than those at the side vaults, they found no significant effect of temperature.

2.3 Persistence

Persistence is the period of time a pathogen or its larval stage can survive in the environment under natural conditions. Generally, persistence is affected by climatic conditions chief among which are temperature, moisture content, location characteristics. Table 8a-c presents the reports of various workers in different parts of the world on the persistence of *Ascaris* ova of different species. Clearly, temperature has an overwhelming effect on the persistence of *Ascaris* ova in different media. Generally, persistence decreases with increasing temperature (Yadav, 2003; de Faria et al., 2017). This is a clear indication that *Ascaris* eggs are more likely to survive longer in the temperate environment than in the tropical environment (Katakam et al. (2013). For instance, at temperatures ≤20°C more than 50% of *Ascaris* eggs in various environmental media could survive for a period ranging from 200 to more than 520 days. Liquid slurry appears to have the most protective effect on *Ascaris* eggs. For an example, while at 5°C the T50 and T99 of eggs in raw slurry were less than 300 and 560 days respectively, the corresponding values for eggs in liquid slurry were > 500 and 1000 days respectively (Katakam et al., 2014a). Similar findings were reported by Strauch (1991). The single data on moisture content/relative humidity provided unequivocal evidence that persistence increased with increasing moisture content/relative humidity at constant temperature. There is about a threefold increase in persistence when moisture content was increased from 40% to 60%, and a whopping 4-5 fold rise in a moisture content increase from 60% to 80%. Although, a no change in persistence when moisture content was increased from 80% and 100% may be interpreted as a tabling effect, such conclusion must be drawn with caution because the researchers simply aborted further observations after 210 days. Besides, a recent investigation in Kwazulu Natal, revealed that under natural conditions *Ascaris* eggs may persist and remain viable in faecal sludge longer than previously imagined. The researchers actually found large numbers of viable *Ascaris* eggs in 15 year old sludge that had spent 2 years buried outside the original pit.

Table 8a. Persistence of *Ascaris suum* eggs in various slurries

Matrix	Temperature °C	T99 (Days) (T50 Days)	Reference
Raw slurry	5	553 (281)	Katakam et al., 2014a
Raw slurry	5	355 (183)	Katakam et al., 2014a
Liquid slurry	5	1034 (519)	Katakam et al., 2014a
Liquid slurry	25	274 (143)	Katakam et al., 2014a
Dungstead manure	40	63	Strauch, 1991
Dungstead manure	50	19	Strauch, 1991
Liquid manure	8	85	Strauch, 1991
Liquid manure	18	65	Strauch, 1991
Slurry (Storage pit)	20+	75	Strauch, 1991
Slurry (Storage pit)	26	28	Strauch, 1991
Liquid manure (Storage pit - aerated)	NR[a]	365	Strauch, 1991
Pig manure (storage pit – non aerated)	NR	365	Strauch, 1991
Sewage sludge (dried)	NR	1825	Strauch, 1991
Sewage sludge (storage pit)	NR	810	Strauch, 1991
Cattle slurry (aerobic)	35 to 40	14	Strauch, 1991
Cattle manure (storage pit)	22 to 27	57	Strauch, 1991
Cattle manure (anaerobic)	55	1	Strauch, 1991
Liquid manure (Licorn-system)	53	2	Strauch, 1991
Pig and cattle manure (anaerobic digestion)	52 to 54	1	Strauch, 1991
Pig slurry (anaerobic thermophilic treatment)	55	1	Strauch, 1991

[a]NR: Not Reported

Table 8b. Persistence of *A. suum* and *A. galli* in Various Slurries (Source: Katakam et al., 2014b)

Matrix[a]	Temperature °C	*A. suum* T50 (Days) Mean (Range)	*A. galli* T50 (Days) Mean (Range)
Slurry with urea	20	42.3 (40.0 to 44.5)	14.1 (9.5 to 18.7)
Slurry with urea	30	21.8 (17.2 to 26.4)	1.22 (0.86 to 1.56)
Slurry with urea	40	0.83 (0.67 to 0.99)	0.05 (0.03 to 0.07)
Slurry with urea	50	0.06 (0.05 to 0.06)	0.01 (0.01 to 0.01)
Slurry without urea	20	242.6 (167.6 to 317.6)	24.8 (16.7 to 32.9)
Slurry without urea	30	41.6 (29.4 to 53.8)	3.81 (3.61 to 4.01)
Slurry without urea	40	NR[b]	0.42 (0.32 to 0.51)
Slurry without urea	50	0.08 (0.07 to 0.08)	0.04 (0.03 to 0.04)

[a]Moisture content/ Relative Humidity (%) was not reported; [b]NR: Not Reported

Table 8c. Persistence of *A. suum* with Varying Relative Humidity in Biosolids at Temperatures between 20-30°C

Moisture Content/ Relative Humidity (%)	T99 (Days)	Reference
30 to 80	49	de Faria et al., 2017
40	15 to 18	Yadav, 2003
60	30 to 56	Yadav, 2003
80	210+	Yadav, 2003
100	210+	Yadav, 2003

3.0 Reductions in Numbers and Viability of Ova by Sanitation Management

Research on sanitation facilities across the world had been centred not only on elucidating factors that speed up decomposition and dehydration, but also on how to ensure complete inactivation of *Ascaris* eggs in different media (Browell and Nelson, 2006; Espinoza et al., 2010). A number of environmental factors found to speed up these processes include temperature, moisture, ventilation, pH, urea and ammonia (Esrey et al., 1998; Pecson et al., 2007). The various investigations assessed not just the efficacy of these factors in the natural environment, but also the effects of artificial variability in their concentrations. In this section, we review the levels of *Ascaris* eggs inactivation in the different types of latrines and materials.

3.1 Treatment of Excreta and Wastewater

3.1.1 Onsite sanitation

3.1.1.1 Dry onsite sanitation systems

All waterless sanitation technologies are on-site. They utilize either the decomposition or dehydration process and produce either compost or biochar as end product (Tables 8 and 9). Decomposition composting sanitation is either urine diverting or non-urine diverting while all dehydrating sanitation are urine diverting. This is because dehydration works better when urine is separated from faeces (Wolgast, 1993; Esrey et al., 1998). The ability of these sanitation systems to inactivate *Ascaris* eggs depends largely on the length of storage and the type of treatment employed to speed up the drying process (Esrey, 1998; Nelson and Darby, 2001).

Table 9. Characteristics of decomposing waterless sanitation devices used in different parts of the world producing compost products (source: Esrey et al., 1998)

Origin	Year	Device	Location	Number of Vaults /Chambers	Design	Processing Time (Months)
India	NR[a]	Double vault toilet	Above land	2	UD[b]	12
Kiribati Is	NR	Movable bin Toilet	Above land	2	NUD[c]	12
Mexico	1970	Sirdo Seco toilet	Above land	2	NUD	12
Norway	1972	Carousel toilet	Below land	4 to 6	UD	12
South Pacific	1992	CCD toilet	Above land	2	NUD	12
Sweden	1960	Clivus Multrum toilet	Above land	1	NUD	12

[a]NR: Rot Reported; [b]UD: Urine diverting latrine; [c]NUD: Non urine diverting latrine

3.1.1.1.1 Inactivation by storage

Faecal sludge can be stored in several ways which can broadly be divided into what may be termed “active sludge storage (ASS)” and “inactive sludge storage (ISS)” systems. ASS concerns faecal heap that is still receiving fresh faeces while ISS refers to heaps no longer receiving fresh faeces (Montangero and Strauss, 2002; Strande et al., 2014; Niwagaba et al., 2014). Thus, ISS are sludge heaps that have been removed from either one of twin vaults in double vault toilets or dug up from single vault drop and store toilets or scooped from drop and transfer systems and stored (Dodane et al., 2011). The storage may be above ground and using different types of containers or underground pits (Pompeo et al., 2016). Irrespective of the manner of storage, the objective is always to allow composts to properly form and achieve pathogen die-off before it is used for agricultural purposes.

Studies have shown that *Ascaris* eggs remain viable in sludge and the environment longer than most other pathogens, and take longer to inactivate (Kaneshiro and Stern, 1986; Jensen et al., 2009). According to Pecson et al. (2007) and Wharton (1980) the persistence of *Ascaris* eggs in the environment can be explained by the characteristics of its shell which is highly impermeable and has been deemed “one of the most resistant biological structures”. For this reason, *Ascaris* egg has been said to be hardy and a standard for assessing any environmental treatment that is not a pre-approved EPA pathogen reduction technique (US EPA, 1999). An EPA approved technique must be able to inactivate *Ascaris* eggs below public health importance. Based on this, the various waterless sanitation technologies must prove that their storage design specifications and functional capacities are able to inactivate *Ascaris* eggs. The period required to achieve this goal vary substantially around the world and significantly depends on prevailing ambient temperature of an area. It is shorter in tropical zones than temperate areas (Kim et al., 2012). For instance, 2-3 years are required in the temperate environment but only 3-12 months in the tropical climate (Tables 9 and 10).

Table 10. Characteristics of dehydrating above land waterless sanitation devices used in different parts of the world (Source: Esrey et al., 1998)

Area	Years of Study	Device	Number of Vaults	Design	Processing Time (Months)	Product	Absorbent /Energy	Anal Cleaning Dropped
Ecuador	1985	Double vault	2	NUD[a]	NR[b]	Biochar/ Compost	Solar heater, ash, sawdust	NR
El Salvador	1994	Tecpan	1	UD[c]	3	Biochar/ Compost	Solar heater, ash, soil/lime	No
Guatemala	1978	Lasf[d]	2	UD	12	Compost	Ash, soil, sawdust, lime	No
India	NR	Ladakh toilet	NA[e]	NUD	NR	Compost	Soil, ash	No
Sweden	1980	WM Ekologen	NR	UD	6	Compost	Ventpipe, fan	Yes
Vietnam	NR	Double vault	2	UD	2	Compost	Ash, soil	No
Yemen	NR	Long drop	NR	UD	NR	Biochar	Sunlight	No

[a]NUD: Non urine diverting latrine; [b]NR: Not reported; [c]UD: Urine diverting latrine; [d]Lasf: Letrina Abonera Seca Familiar Latrine; [e]NA: Not Applicable; Other factors reported to speed up inactivation include ammonia (Nordin et al., 2009; Katakam et al., 2014a), length of storage (Pompeo et al., 2016), the physico-chemical conditions of the bedding materials (Katakam et al., 2014b) and temperature.

This means that compost from ASS system from all sanitation facilities must not be used immediately but must be subjected to a reasonable period of inactive storage state before use (Gibson, 2014). The period of inactive storage should normally be longer in temperate than tropical climate zones (Gibson, 2014).

Table 11 presents the direct effect of temperature variability on *Ascaris* egg inactivation in any given area. Clearly it can be deduced that for every twofold increase in temperature the period required to completely inactivate *Ascaris* eggs is halved. That is, increasing temperature leads to increasing rate of *Ascaris* egg inactivation. However, care must be taken in interpreting the mixed data on activation from different sources with different dates and using different analytical procedures. Besides the different methods used to measure the egg inactivation may have different sensitivity and precision, thus raising a risk in cross comparison.

Table 11. Effect of temperature on the time for inactivation of *Ascaris* eggs

Matrices	Temperature °C	Time for 100% Inactivation (Days)	Reference
Faecal sludge	<20	365 to 730	Carr, 2001
Faecal sludge	≥20	<365	Carr, 2001
Faecal sludge	<20	Several months	Schönning and Stenström, 2004
Faecal sludge	20 to 30	Several months	Schönning and Stenström, 2004
Faeces	<20	50 to 200	Ambjerg-Nielsen et al., 2003
Night soil	20 to 30	Many months	Ambjerg-Nielsen et al., 2003
Sludge composting	Ambient	Many months	Ambjerg-Nielsen et al., 2003
Sludge composting	50 to 60	7	Ambjerg-Nielsen et al., 2003
Waste stabilization pond (retention time >20 days)	Ambient	20	Ambjerg-Nielsen et al., 2003
Soil	<20	15 to 100	Ambjerg-Nielsen et al., 2003
Soil	Ambient	Many months	Esrey et al., 1998

3.1.1.1.2 Pit latrines, vault toilets, dry toilets

All waterless drop and store sanitation models can be described as dry toilets (Windberg, 2009). They include the traditional pit (TP) latrine, conventional improved pit (CIP) latrine, slit-trench (ST) latrine, very improved pit (VIP) latrine and vault latrines (Esrey et al., 1998). They however, differ in the type of containment used to store faeces. The pit latrines which range from TP to VIP store faeces in dug out pits, ideally 4-5 meters deep. The pits may or may not be lined with concrete and maybe single or double (Mahenge, 2013). In double pit system, only one is used at a time during which the other is covered. When one is full, the second is opened for use and the full one closed for two or three years before it is evacuated and the compost so formed used as fertilizer (Austin, 2006). Vault toilets use vault boxes of different shapes and sizes (Esrey et al., 1998). Like pit latrines, there are single and double vault models used in the same manner. However, unlike the pit latrine, vault latrines are moveable. Thus, when one vault in a double vault is full, it is removed and stored for 2-3 years before the compost is formed then after that it is used for fertilizer in agricultural farms (Jensen et al., 2009; Vinneral et al., 2009). The composting latrines are basically vault latrines into which desiccants such as ash, sawdust or soil is added. The major processing method in pit and vault latrines is decomposition. According to Uno and Simpson-Hebert (2004) pit sludge at any given time decomposes in both the aerobic and anaerobic systems. Aerobic activity takes place near the surface called aerobic zone while anaerobic activity takes place at deeper profile known as anaerobic zone (Niwagaba et al., 2014). Many factors, especially pit and tank design affect the boundary between aerobic and anaerobic zones (Mehl, 2008).

Decomposition kills *Ascaris* eggs but a substantial number still survives for years. In twin pit and double vault models, storage of one after it is full means that no fresh faeces goes into it and this allows pathogen inactivation and decomposition of the excreted material to continue undisturbed (concept of ISS) (Berendes et al., 2015).

Table 12 summarizes the outcome of several investigations on the level of inactivation and persistence of *Ascaris* eggs under several conditions. Clearly rate of inactivation is affected by several factors singly or in combination. Working independently, Carr (2001) and Schönnlng and Stenström (2004) showed that rising temperature speeds up rate of inactivation. While Carr (2001) revealed that time taken to achieve a 2 $\log_{10}$ reduction in *Ascaris* eggs in faecal sludge is halved for every 10°C rise in temperature, Schönnlng and Stenström (2004) found at least a fourfold reduction at the same level of rise in temperature. Treatment with either quicklime (CaO) or ammonia (NH_3) had no effect at 20°C or less but speeded-up *Ascaris* egg die-off from temperatures 25°C and above. Other additives found to speed up inactivation included $Ca(OH)_2$ and $(NH_4)_2SO_4$. The effectiveness of each of these additives increased with rising temperature. Pecson et al. (2007) investigated the specific effects of varying temperature, pH and ammonia concentrations on the time it took to inactivate 2 $\log_{10}$ (i.e. T99) of *Ascaris* ova in faecal sludge. Varying pH from 7 to 12 at 20°C reduced by almost one half the time taken (430 to 230 days). Addition of 1000 and 5000 mg/L of NH_3 further reduced the time threefold and fivefold respectively. The same trend was seen with every increase in temperature except that at 50°C change in pH had no further effect and time taken to achieve T99 came down to minutes rather than hours and days.

Table 12. Inactivation of *Ascaris* eggs in faecal sludge temperature and treatments

Treatment	Temperature °C	pH	Removal/ Inactivation level (Log_{10})	Time[a] (Days)	Reference
NR	<20	NR[c]	>2	750 to 1095	Carr, 2001
NR	20 to 30	NR	>2	<365	Carr, 2001
NR	<20	NR	>2	50 to 200	Schönnlng and Stenström, 2004
NR	20 to 30	NR	>2	Several months	Schönnlng and Stenström, 2004
NH3 (10-50%)	20	NR	<1 to 2	2h[b]	Mendez et al., 2002
CaO (5-40%)	20	NR	<1 to 2	2h	Mendez et al., 2002
Ca(OH)2	NR	NR	<2	I	Gantzer et al., 2001
CaO	NR	NR	<2	1	Gantzer et al., 2001

Treatment	Temperature °C	pH	Removal/ Inactivation level (Log_{10})	Time[a] (Days)	Reference
NaOH+1% (w/v) (NH4)2SO4	37	NR	2	2	Kato et al., 2003
Ca(OH)2	25	NR	<2	7	Plachy et al., 1996
NaOH+50 mg/g TS of (NH4)2SO4	Ambient	NR	<2	10	Reimers et al., 1986a
NH4OH	22	NR	2	21	Ghiglietti et al., 1997
CaO	NR	NR	<2	40	Brewster et al., 2003
CaO	45	NR	2	70	Eriksen et al., 1996
$Ca(OH)_2$	Ambient	NR	<2	98	Schuh et al., 1984
NH_3 mg/l	20	7	2	450	Pecson et al., 2007
None	20	12	2	230	Pecson et al., 2007
1000	20	12	2	87	Pecson et al., 2007
5000	20	12	2	25	Pecson et al., 2007
None	30	7	2	180	Pecson et al., 2007
None	30	12	2	24	Pecson et al., 2007
1000	30	12	2	16	Pecson et al., 2007
5000	30	12	2	4.8	Pecson et al., 2007
None	40	7	2	14	Pecson et al., 2007
None	40	12	2	3.4	Pecson et al., 2007
5000	40	12	2	9.4h	Pecson et al., 2007
None	50	7	2	110 min[d]	Pecson et al., 2007
None	50	12	2	120 min	Pecson et al., 2007
5000	50	12	2	97 min	Pecson et al., 2007

[a]Time in days unless indicated otherwise; [b]h: hours; [c]NR: Not Reported; [d]min: minutes

3.1.1.1.3 Composting

The final product of all drop and store latrines is the compost which can be used to fertilize agricultural crops (Table 13). Composts are assessed on the basis of their quality and safety. Quality is the ability of composts to condition soils for better agricultural production, while safety is assessed by the presence and number of pathogens especially *Ascaris* ova (Magri et al., 2013; Gibson, 2014). Thus, good quality compost must not only be a good soil conditioner for better agricultural production, it must also not contain pathogens that constitute risks to human health.

According to Carr (2001) several factors determine the quality and safety of compost produced by a latrine. Among these factors are the ability to support biological decomposition of sludge, high oxygen tension that is sufficient and able to penetrate the compost heap to maintain aerobic conditions, moisture content of 50-60%, carbon:nitrogen ratio (the C:N ratio) in the range of 15:1 to 30:1 and temperature of 20°C and above. In addition, the compost must provide necessary conditions for complete pathogen die-off. Naturally, a variety of organisms contribute to the breakdown of the material in a composting pit. They range in size from viruses, bacteria, fungi and algae to earthworms and insect larvae. All of these play a major role in mixing, aerating, tearing apart and breaking down the contents of the pile in the toilet's processing vault (Gibson, 2014). As long asthey remain inside the vault their activities are good and should be encouraged. It might even be a good idea to place earthworms in the toilet (Nakagin et al., 2016). If the environment is favourable for them they will multiply, burrowing holes through the compost heap, eating odorous organic matter and thereby converting same to rich organic soil.

Table 13. Inactivation of *Ascaris* eggs in compost of varying ages, pH, moisture content and desiccant treatments

Area	Matrix[a]	pH	Desiccant	Removal/inactivation Mean (Log_{10})	Time (Days)	Reference
China	Compost (from UDDT[b])	9 to 10	NR[c]	2	120	Wang, 1999
China	Compost (from UDDT)	>8	NR	.2	120	Lan et al., 2001
El Salvador	Compost (from 118 UDDT; 38 single vault)	<11	Ash, lime or lime+soil	2	700	Wang, 1999
El Salvador	Compost (from 118 UDDT; 38 single vault)	>11	Ash, lime or lime+soil	2	450	Wang, 1999
Vietnam	Compost (from 12 UDDT) Moisture 24 to 55%	8.5 to 10.3	Ash	1.3 to > 2	63	Carlander and Westrell , 1998

[a]Age of compost 4 to 10 months; [b]UDDT: Urine-diverting dry toilet; [c]NR: Not Reported

In contrast, helminth parasites, especially the faeces and urine borne, e.g. *Ascaris* contribute nothing to the process of composting but are important constituent of human excreta discharged into the latrines (Niwagaba et al., 2014). The safety of composts is thus defined by the degree of die-off among these and other pathogenic

organisms before a compost is used. Usually, as already stated, dynamic activities in the latrines can achieve complete pathogen die-off in compost within 365 to 730 days depending on prevailing conditions. However, the period taken to achieve complete pathogen die-off can be speeded up by artificial treatment of compost. Some of these treatments as discussed in the preceding sections and shown in Table 13 speed up pathogen die-off and achieve complete or near complete (e.g. T99) *Ascaris* egg inactivation faster than natural processes.

These include increasing temperature, exposure to sunlight and increasing the pH, on the one hand, and decreasing moisture content, nutrient concentration and microorganism density on the other hand. Studies suggest that optimum pH lies above 11. For instance, at pH <11 (and all other factors kept constant) Wang (1999) achieved complete *Ascaris* inactivation in 700 days. At pH >11 however, and under similar conditions, same level of inactivation was achieved in 450 days, which was 250 days faster.

3.1.1.2 Water-based onsite sanitation (septic tanks)

The septic tank is the receptor and storage device component of the water closet sanitation system. Though an onsite sanitation system, it is split into indoor and outdoor components. The indoor component comprises the water cistern (for water storage) and the bowl (for defaecation). The two components are connected via a pipe network. The septic tank is watertight and receives faeces and flush water (blackwater) from toilets and household sulllage (grey water) from the kitchen (Carr, 2001). Although the faecal sludge in septic tanks can be stored for several years depending on the quantity of faeces per unit time and size of septic tank, it is periodically removed and treated off-site. The offsite treatment is associated with the risk of viable helminth eggs contaminating the environment. The most important of such helminths is the *Ascaris* which as has been noted earlier is highly resilient (Jensen et al., 2009; Muller et al., 1989). While only a limited report is available in the literature, the few that can be assessed showed that more than half of *Ascaris* eggs in septage of septic tanks can remain viable for over two years (Table 14). Number of eggs in septage may range from 25 to over 4000 depending on the health status and number of users (Gallizzi, 2003; SANDEC/EAWAG-IWMI, 2003; Jimenez-Cisneros and Maya-Rendon, 2007).

Table 14. Number and viability of *Ascaris* eggs in fresh sludge, septage and biosolids

Matrix	Age	Number of Eggs/g	Egg Viability (%)	Reference
Fresh sludge (Ghana)	Fresh	35 to 2.32E+02	NR[a]	Jimenez- Cismeros and Maya-Rendon, 2007
Septage (Country not reported)	>2 yrs old	5.80E+02	45 to 82	Jimenez- Cismeros and Maya-Rendon, 2007
Septage (Ghana)	NR	9.00E+02 to 6.9E+03 (n=7)	NR	Gallizzi, 2003; SANDEC/EAWAG-IWMI, 2003
Biosolids (Ghana)	NR	20 to 85 (n=8)	45 to 82	Gallizzi, 2003; SANDEC/EAWAG-IWMI, 2003
Septage (Country no reported)	NR	± 4.0E+03	NR	Strauss, 1994; Mara and Cairu, 1989; Montangero and Strauss, 2002

[a]NR: Not Reported

3.1.3 Wastewater lagoons and stabilization ponds

Table 15 presents the effects of the processes and varying treatment options on the inactivation of *Ascaris* eggs in wastewater ponds.

Table 15. Inactivation of *Ascaris* eggs in sewage of different treatment processes in waste stabilization pond

Pond Type[a]	Treatment	Removal/Inactivation Level (Log_{10})	Reference
Waste stabilization pond[a]	UV	1	Fuhrmann and Rudolph, 2009
Waste stabilization pond[a]	Biological	0.66 to >2	Okojokwu and Inabo, 2012; Awuah, 2006
Waste stabilization pond[a]	Dialysis chambers	0.3 to 0.4	Nelson and Darby, 2002
Waste stabilization pond[a]	Sludge cores	0.3 to 0.4	Nelson and Darby, 2002
Waste stabilization pond[a]	Biological (14 days)	0.83±0.4	Shanthala et al., 2007
Waste stabilization pond[a]	Biological	1.3	Shanthala et al., 2007
Facultative lagoon	Biological	2.4	Quiroga, 2011 Reinoso et
Facultative lagoon	Biological	1.12	al., 2008
Facultative lagoon	Biological	0.82 to 1.3	Hays, 1977; Jimenez-Cismeros and Maya-Rendon, 2007
Facultative lagoon	Biological (12 days)	>2	Heinss et al., 1998; Montangero and Strauss, 2002

[a]Number of ova per litre of water in developing regions 70 to 3000 and in developed regions 1 to 80 (Jimenez-Cismeros and Maya-Rendon, 2007)

3.1.4 Wastewater treatment and resource recovery facilities

Both the traditional and emerging treatment options aim at removing substantial portions of contaminants and pollutants in wastewater to produce waste stream or solid waste suitable for discharge or reuse. The methods of wastewater treatment are several but can broadly be classified into physical, chemical or biological. They function by mechanical removal of solids, aerobic and anaerobic oxidation, chemical flocculation and sedimentation etc. According to Jimenez-Cisneros and Maya-Rendon (2007) helminth ova including *Ascaris* ova in wastewater are particles forming a fraction of suspended solids. As a result mechanisms used to remove suspended solids are also used to remove helminth ova from wastewater (Reimers et al., 1986a, Reimers et al., 1986b). These mechanisms according to the authors are sedimentation, filtration and coagulation-flocculation. However, specific investigations to assess the ability of these methods to inactivate *Ascaris* ova in wastewater are lacking in the literature.

3.1.5 Biosolids management

The most important pathogen in biosolids is the *Ascaris* ova because it persists in the environment longer than other pathogens. Safe use of biosolids to fertilize agricultural farms depends on how much of these pathogens are present and how much made could be inactivated before use. Although theoretically, the process of producing biosolids is on its own capable of killing these pathogens, the process is slow and for Ascaris ova it is even slower (Pecson et al. (2007). This is why contact with inadequately treated biosolids is associated with an increased risk of *Ascaris* infection (Corrales et al., 2006). This may explain why prevalence of *Ascaris* infection is usually higher in areas where biosolids is commonly used in agriculture than in areas where they are not.

3.2 Disinfection

Sanitisation and disinfection are common words used in wastewater and pathogen management. Both are processes used to reduce microbes and parasites in the environment. Sanitisation technologies were those originally that were known to provide cleanliness and reduction of some level of pathogens mostly bacteria (this was based on experience at the time and not risk or quantitative assessment). Disinfection is based on the use of physical and chemical agents (eg UV and chlorine, ammonia). Sanitizing agents shall be adequate and safe under conditions of use. Any facility, procedure, or machine is acceptable for cleaning and sanitizing equipment and utensils if it is established that the facility, procedure, or machine will routinely render equipment and utensils clean and provide adequate cleaning and sanitizing treatment.

Disinfection uses antimicrobial agents on non-living objects or surfaces to destroy or inactivate microorganisms. Disinfectants may not kill all bacteria, viruses, fungi and spores. Most disinfectants are weakened or inactivated by organic matter such as dirt and feces. Sanitation uses an antimicrobial agent on objects, surfaces or living tissue to reduce the number of disease-causing organisms to non-threatening levels. Sanitizing does not affect some spores and viruses. A practical method of sanitizing hands is to wash them with soap under running hot water for at least 20 seconds. Sterilization is using chemicals, temperature, gas and/or pressure to kill or inactivate all disease-causing bacteria, spores, fungi and viruses.

3.2.1 Physical processes for the inactivation of *Ascaris*

Table 16 presents the effects of various approaches for the inactivation of *Ascaris* eggs in different types of faecal matter. The table suggests that many processes have the ability to achieve greater than two or more $\log_{10}$ reduction in *Ascaris* ova at varying periods of time. Anaerobic digestion under thermophilic condition which appeared to be the most effective technique achieved a 100% reduction within 4 hours. Composting achieved a similar result but at different periods of time depending on the location of *Ascaris* eggs in the compost heap. While eggs at the front and back of compost heap were totally inactivated in two days, those at the centre were inactivated half a day faster. The other processes such as exposure to low pressure UV and heat treatment took longer, sometimes up to 14 days (Ligocka and Paluszak, 2009).

Table 16. Effects of sanitisation processes on the inactivation of *Ascaris* ova in different types of faecal materials

Matrix	Primary Process	Medium/Exposure Pattern	Exposure Duration (Days)	Days to Achieve > 2 log_{10} Reductions	Reference
Organic waste	Composting	Front of heap	NR[a]	2	Ligocka and Paluszak, 2009
Organic waste	Composting	Middle or centre of heap	NR	1.5	Ligocka and Paluszak, 2009
Organic waste	Composting	Back of heap	NR	2	Ligocka and Paluszak, 2009
Organic waste	Fermentation	Mesophilic	NR	14	Ligocka and Paluszak, 2009
Organic waste	Fermentation	Thermophilic	NR	1	Ligocka and Paluszak, 2009
Slurry	Anaerobic digestion	Thermophilic bioreactor (Exp 1 & 2)	NR	4h	Paluszak et al., 2016
Slurry	Low pressure radiation	UV Jm2	250[b]	10[b]	Raynal et al., 2012
Slurry	Low pressure radiation	UV Jm2	500 to 750	10	Raynal et al., 2012
Slurry	Low pressure radiation	UV Jm2	1000[c]	10[c]	Raynal et al., 2012
Slurry	Low pressure radiation	UV Jm2	10000[b]	10[b]	Raynal et al., 2012
Slurry	Heat Treatment	70°C	30s[d] to 3min[e]	10	Raynal et al., 2012
Slurry	Heat Treatment	48°C	6 to 24h[f]	10	Raynal et al., 2012
Slurry	Low temperature and ammonia	42°C; pH 9; 2,000 mg/l NH_3-N	18 to 72h	10	Raynal et al., 2012

[a]NR: Not Reported; [b]<2 log_{10} reductions at 10 days; [c]<1 log_{10} reductions at 10 days; [d]s: seconds; [e]min: minutes; [f]h: hours

3.2.2 Chemical Disinfection

Disinfectants are applied both for preventive and control purposes. At recommended concentrations they are active against microbes, parasites, insects of different sizes and their respective larval stages. Several are for household use while many others are used to disinfect water, surfaces and materials in industries, institutions and several other environments (Oh et al., 2016). Table 17 presents the effect of some common disinfectants on the embryonation and inactivation of *Ascaris* ova. In a study carried out in Korea, Oh et al., (2016) reported that after 3 weeks of incubation following exposure to ethanol and methanol there was no larval development of decorticated *A. suum* eggs at all concentrations and exposure time. Chlorohexidin (5%) also had almost no effect on embryogenesis of *A. suum* eggs In contrast, 10% povidone iodine, 3% cresol, 0.2% sodium hypochlorite, and 0.02% sodium hypochlorite inhibited embryonation at 3 weeks of incubation. At 6 weeks of incubation, however, eggs exposed to all disinfectants tested showed embryonation regardless of exposure time, except for 10% povidone iodine. Thus, only 10% povidone iodine completely inhibited the embryonation of decorticated *A. suum* eggs at 6 weeks of incubation. In another study in South Africa, Naidoo et al. (2016) investigated the effectiveness of four common disinfectants (sodium hypochlorite with and without detergent, carbolic acid and an unknown compound) to inactivate *Ascaris* ova on different surfaces and media. According to their report, exposure to 50% of these disinfectants led to a marked decline in the number of viable eggs. Although sodium hypochlorite proved based disinfectants were more effective than the others but none attained up to 2 $\log_{10}$ reduction (Table 17). The researchers also reported that eggs exposed to disinfectants appeared decorticated and the chitinous layer was removed.

Table 17. Ability of different disinfectants to inactivate and inhibit embryonation of *Ascaris* eggs

Disinfectants	Dilution Level (%)	Treatment Duration (Min)	Removal / Inactivation Level (Log_{10})		Reference
			3 Weeks[c]	6 Weeks[c]	
Ethanol	70[a]	0.5 to 60	0	0	Oh el al., 2016
Ethanol	99[a]	0.5 to 60	0	0	Oh et al., 2016
Methanol	70[a]	0.5 to 60	0	0	Oh et al., 2016
Methanol	99[a]	0.5 to 60	0	0	Oh et al., 2016
Povidone iodine	10[a]	0.5 to 60	>3	>3	Oh et al., 2016
Cresol	3[a]	0.5 to 60	0 to >2	0	Oh et al., 2016
Sodium hypochlorite	0.02[a]	0.5 to 60	0 to >2	0	Oh et al., 2016
Sodium hypochlorite	0.2[a]	0.5 to 60	0 to >2	0	Oh et al., 2016
Chlorohedidin	0.2[a]	0.5 to 60	0 to <1	0	Oh et al., 2016
Sodium hypochlorite with detergent	50[b]	1 to 12	<1 to >1	>1	Naidoo et al., 2016
Sodium hypochlorite without detergent	50[b]	1 to 12	<1 to >1	>1	Naidoo et al., 2016
Carbolic acid	50[b]	1 to 12	<1	<1	Naidoo et al., 2016
Unknown compound	50+[b]	1 to 12	<1	<1	Naidoo et al., 2016

[a]Diluent was distilled water; [b]Diluent was tap water; [c]Periods of assessment

3.2.3 Ammonia

Among all disinfecting chemical compounds identified as having ovicidal effects against Ascaris eggs, ammonia appears to be the best. Apart from its effectiveness when used singly, ammonia also helps to quicken the action of other agents of Ascaris inactivation in faecal materials. Several studies on the effectiveness of ammonia as an agent of Ascaris ova inactivation abound in the literature and some of the most recent are presented in Table 18a-d. Data presented show clearly that the rate of ammonia inactivation of Ascaris eggs increases with increasing pH. For instance, at pH 6.8 and ammonia concentration of 2098 mg/L, a 2 $\log_{10}$ inactivation was achieved in 160 days. By increasing the pH to 10.4 an ammonia concentration of 2637 achieved the same level of inactivation in just 52.5 days which was threefold faster (McKinley et al., 2012). A similar report by Pecson et al. (2007) showed that at pH 7 and no ammonia a 2 $\log_{10}$ inactivation was achieved in 450 days at 20°C. Increasing the pH to 12 at the same temperature and zero ammonia, the same level of inactivation was attained in 230 days which was half the time taken at pH 7. By introducing 1000 mg/L of ammonia at the same pH 12 and temperature of 20°C a 2 $\log_{10}$ inactivation was achieved within 87 days. The time was further reduced to mere 25 days by increasing ammonia concentration to 5000 mg/L. By increase the temperature at the same levels of pH and ammonia, inactivation time was further reduced. For instance, at 50°C, 5000 mg/L ammonia concentration and pH 12, a 2 $\log_{10}$ inactivation was achieved within 7 hours. Thus, ammonia activity was exacerbated by increasing pH and temperature (Pecson et al., 2007; Nordin et al., 2009; Raynal et al., 2012).

Table 18a. Ammonia inactivation of *Ascaris* ova in urine and varying pH and temperature

Matrix	Temperature °C	pH	Ammonia (mg/L)	Time (Days) For 2 Log_{10} Removal/ Inactivation	Reference
Stored urine + ash	19.5	10.4	2637	52.5	McKinley et al., 2012
Fresh urine	19.5	6.8	2098	159.6	McKinley et al., 2012
Fresh urine + ash	19.5	10.4	540	104.3	McKinley et al., 2012
Urine at 1:0 dilution	14	9.0 to 9.1	236	240	Nordin et al., 2009

Matrix	Temperature °C	pH	Ammonia (mg/L)	Time (Days) For 2 Log_{10} Removal/ Inactivation	Reference
Urine at 1:0 dilution	24	9.0 to 9.1	141	48	Nordin et al., 2009
Urine at 1:0 dilution	34	9.0 to 9.1	141	3.4	Nordin et al., 2009
Urine at 1:0 dilution	NR[a]	9.0 to 9.1	57	480	Nordin et al., 2009
Urine at 1:1 dilution	14	8.9	39	1060	Nordin et al., 2009
Urine at 1:1 dilution	24	8.9	66	56	Nordin et al., 2009
Urine at 1:1 dilution	34	8.9	100	6.3	Nordin et al., 2009
Urine at 1:1 dilution	NR	8.9	16	840	Nordin et al., 2009
Urine at 1:3 dilution	14	8.7 to 9.1	20	NR	Nordin et al., 2009
Urine at 1:3 dilution	24	8.7 to 9.1	18	NR	Nordin et al., 2009
Urine at 1:3 dilution	34	8.7 to 9.1	40	8.5	Nordin et al., 2009
Urine at 1:3 dilution	NR	8.7 to 9.1	14	NR	Nordin et al., 2009
Sludge	44	9 to 11	200	1[b]	Pecson and Nelson, 2005

[a]NR: Not Reported; [b]log_{10} inactivation <1; [c]log_{10} inactivation >2

Table 18b. Inactivation of *Ascaris* ova in sludge and varying Ammonia, pH and temperature

Matrix	Temperature °C	pH	Ammonia (mg/L)	Time (Days) For 2 Log_{10} Removal/ Inactivation	Reference
Stock solution	42	9	2000	10[a]	Raynal et al., 2012
Sludge with urine	24	12.5	6.6	14	Ogunyokun et al., 2016
Sludge with urea	24	8	9.1	14	Ogunyokun et al., 2016
Sludge	20	7	0	450	Pecson et al., 2007
Sludge	20	12	0	230	Pecson et al., 2007
Sludge	20	12	1000	87	Pecson et al., 2007
Sludge	20	12	5000	25	Pecson et al., 2007
Sludge	30	7	0	180	Pecson et al., 2007
Sludge	30	12	0	24	Pecson et al., 2007
Sludge	30	12	1000	16	Pecson et al., 2007
Sludge	30	12	5000	0.39	Pecson et al., 2007
Sludge	40	7	0	14	Pecson et al., 2007
Sludge	40	12	0	3.4	Pecson et al., 2007
Sludge	40	12	5000	0.3	Pecson et al., 2007
Sludge	50	7	0	100 mins[b]	Pecson et al., 2007
Sludge	50	12	0	120 mins	Pecson et al., 2007
Sludge	50	12	5000	97 mins	Pecson et al., 2007
Sludge, 10% TS	22	9.1	2400	90	Ghigletti, 1997
Sludge, 10% TS	22	12	9700	60	Ghigletti, 1997
Sludge, 10% TS	22	12.7	1200	40	Ghigletti, 1997
Sludge, 10% TS	22	12.7	19400	21	Ghigletti, 1997

[a]log_{10} inactivation >2; [b]Mins: Minutes

NOTE: Sludge without urine at temperatures of 24°C pH of 12.3 had very little ammonia (0.38 mg/L) and only 0.38 log_{10} inactivation in 14 days. Sludge with urea at temperatures of 24°C pH of 8.0 also had very little ammonia (9.1 mg/L) and only 0.42 log_{10} inactivation after 14 days.

Table 18c. Inactivation of *Ascaris* ova in faeces and varying Ammonia, pH and temperature (Source: Nordin et al., 2009)

Matrix	Temperature °C	pH	Ammonia (mg/L)	Time (Days) for 2 Log_{10} Inactivation
Faeces+2%urea	24	9	230	28
Faeces+1%urea	24	8.9	130	47
Faeces+ash+1%urea	24	12.8	220	13
Faeces+ash	24	12.8	57	35
Faeces+urea	24	8.3	20	74
Faeces+2%urea	34	9	440	3.8
Faeces+1%urea	34	8.9	250	4.1
Faeces+ash+1%urea	34	12.8	72	3.8
Faeces+ash	34	12.8	71	3.7
Faeces+urea	34	8.3	43	21

Table 18d. Inactivation of *Ascaris* ova in water and varying Ammonia, pH and temperature

Matrix	Temperature °C	pH	Ammonia (mg/L)	Time (Days) For 2 Log_{10} Inactivation	Reference
Deionized water+ash	19.5	10.4	47	175.7	McKinley et al., 2012
Water solution	30	11.9	2900	14	Ghigletti, 1997
Water solution	40	11.9	2900	7	Ghigletti, 1997
Distilled water	22	12.3	2400	90	Ghigletti, 1997
Distilled water	22	12.5	4800	40	Ghigletti, 1997
Distilled water	22	12.7	7300+	21	Ghigletti, 1997

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