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Philip A. Collender Date

Ascaris viability and assessment of risk for a vermicomposting ecological sanitation system in El Alto, Bolivia

By

Philip A. Collender
Master of Public Health

Global Environmental Health

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Christine L. Moe, PhD
Committee Chair

_____

Paige Tolbert, PhD
Committee Member

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By

Philip A. Collender

B.S., University of Virginia, 2009

Thesis Committee Chair: Christine L. Moe, PhD

An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Global Environmental Health
2013

Abstract

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By Philip A. Collender

An estimated 64 million Disability Adjusted Life-Years (DALYs) are lost to diseases caused by unsafe water, poor sanitation and poor hygiene every year, mostly in developing countries. Ecological sanitation (Ecosan) provides low-cost sanitation appropriate for many developing regions, funded through the sale and reuse of excreta for horticultural applications. Reuse of incompletely sanitized excreta may result in transmission of infectious agents, especially the ova of helminths such as *Ascaris lumbricoides*. Composting with earthworms, or vermicomposting, is a potential sanitization technique, but has received little formal study. To address this knowledge gap and provide guidance for a Bolivian NGO managing an Ecosan system in El Alto, Bolivia, *Ascaris* ova were quantified in samples of vermicomposts after 3, 6, 8, 13, or 18 months of composting. Bayesian models of inactivation of *Ascaris* in the vermicomposts estimated 97.5th percentile times for 90% inactivation of thousands of months, and did not indicate statistically significant decay over time. Best-case estimates of the median annual burden of disease for consumers of raw produce fertilized with vermicompost ranged from $1.64*10^{-5}$ to $8.25*10^{-2}$ DALYs/person/year, depending on produce type and the dose-response model used. Best-case estimates of the median annual burden of disease for agricultural workers laboring on plots fertilized with vermicompost ranged from $6.05*10^{-7}$ to $1.98*10^{-2}$ DALYs/person/year. Best-case estimates of the median annual burden of disease for children playing in parks fertilized with vermicomposts ranged from $9.05*10^{-4}$ to $6.84*10^{-2}$ DALYs/person/year. Estimated burdens of disease for most scenarios did not fall below 10^{-4} DALYs/person/year unless the concentration of viable *Ascaris* ova in the vermicompost was at levels undetectable (≤0.25 ova/g total solids) by the USEPA method for *Ascaris* detection in biosolids. Due to the unacceptably high burdens of disease estimated for children and consumers of produce, even when ova concentrations are below the limit of detection, a conservative recommendation to protect public health is to restrict application of biosolids from public spaces and crops that might be eaten raw unless helminth infections are known to be extremely rare in the population. Future research is recommended to address assumptions made in the risk models constructed for the present study.

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Acknowledgements:

I would like to thank my faculty advisor, Christine Moe, for connecting me with Sumaj Huasi and providing me with the opportunity to perform this study, as well as for her support and rigor in providing feedback along the way. I must also thank Dr. Juan Leon, who stepped in at times when Dr. Moe was not available and provided much needed guidance and support in completing this work.

I am very grateful to the many individuals who helped me to gather the data and develop the methods needed to conduct this study: Scott Seitz, who drafted laboratory protocols, Henry Bishop, who trained me in the identification of parasite ova, Julia Sobolik, who guided me through Scott's protocols, Claudia Sanchez, who was my laboratory assistant, Drs. Alfredo Terrazas and Raul Silveti of Sumaj Huasi, who showed me the ropes Bolivia-side, Ronaldo of Sumaj Huasi, who helped me fill up plastic bags with wormy Ecosan compost, Dra. Volga Iniguez, who provided me with a space to work in Bolivia even though it got her own limited lab space dirty, and Dr. Peter Teunis, whose modeling prowess is a source of awe and inspiration.

I owe a tremendous debt of gratitude to many of the good people of Sumaj Huasi for caring for me while I was briefly hospitalized. I would also like to extend my heartfelt thanks to my Bolivian and American friends whose companionship and good humor brightened my every day in La Paz. Un abrazo fuerte desde la distancia.

Finally, many thanks to my friends and family stateside who have supported me through the long haul.

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Role

For this manuscript, the author was responsible for data collection, data analysis, writing of all sections, and the development of the tables and figures. Major assistance in creating the Bayesian statistical models for inactivation of *Ascaris* ova over time and for extrapolating additional data points was provided by Dr. Peter Teunis.

Abstract

An estimated 64 million Disability Adjusted Life-Years (DALYs) are lost to diseases caused by unsafe water, poor sanitation and poor hygiene every year, mostly in developing countries. Ecological sanitation (Ecosan) provides low-cost sanitation appropriate for many developing regions, funded through the sale and reuse of excreta for horticultural applications. Reuse of incompletely sanitized excreta may result in transmission of infectious agents, especially the ova of helminths such as *Ascaris lumbricoides*. Composting with earthworms, or vermicomposting, is a potential sanitization technique, but has received little formal study. To address this knowledge gap and provide guidance for a Bolivian NGO managing an Ecosan system in El Alto, Bolivia, *Ascaris* ova were quantified in samples of vermicomposts after 3, 6, 8, 13, or 18 months of composting. Bayesian models of inactivation of *Ascaris* in the vermicomposts estimated 97.5th percentile times for 90% inactivation of thousands of months, and did not indicate statistically significant decay over time. Best-case estimates of the median annual burden of disease for consumers of raw produce fertilized with vermicompost ranged from $1.64*10^{-5}$ to $8.25*10^{-2}$ DALYs/person/year, depending on produce type and the dose-response model used. Best-case estimates of the median annual burden of disease for agricultural workers laboring on plots fertilized with vermicompost ranged from $6.05*10^{-7}$ to $1.98*10^{-2}$ DALYs/person/year. Best-case estimates of the median annual burden of disease for children playing in parks fertilized with vermicomposts ranged from $9.05*10^{-4}$ to $6.84*10^{-2}$ DALYs/person/year. Estimated burdens of disease for most scenarios did not fall below 10^{-4} DALYs/person/year unless the concentration of viable *Ascaris* ova in the vermicompost was at levels undetectable ($\leq$0.25 ova/g total solids) by the USEPA method for *Ascaris* detection in biosolids. Due to the unacceptably high burdens of disease estimated for children and consumers of produce, even when ova concentrations are below the limit of detection, a conservative recommendation to protect public health is to restrict application of biosolids from public spaces and crops that might be eaten raw unless helminth infections are known to be extremely rare in the population. Future research is recommended to address assumptions made in the risk models constructed for the present study.

Literature Review

Lack of access to safe sanitation is a major public health problem worldwide, especially in the developing world. According to a report by the World Health Organization (WHO) and United Nations Children's Fund (UNICEF), in 2010 2.6 billion people, or 37% of the global population lacked access to improved sanitation, while 1 billion people, or 15% of the population, lacked access to any sanitation [1].

The WHO has estimated that 64 million Disability Adjusted Life-Years (DALYs) are lost to diseases caused by unsafe water, poor sanitation and poor hygiene every year, making lack of safe water and sanitation the 4th most important risk factor globally, and the 2nd most important (behind malnutrition) among developing countries [2]. Lack of adequate sanitation puts populations at risk for morbidity and mortality from an array of infectious diseases caused by organisms ranging from bacteria, such as *Vibrio cholerae*, viruses, such as *Rotavirus*, protozoans, such as *Entamoeba histolytica*, to helminths, such as *Ascaris lumbricoides*. While bacterial, viral, and protozoal infections are generally associated with acute morbidity and mortality, helminth infections tend to manifest as chronic diseases of lesser severity. *Ascaris* infections are common, affecting up to 20% of the global population [3], accounting for an estimated annual loss of 10.5 million DALYs [4], and roughly 10,000 deaths per year [5]. Ascariasis and other helminth infections have been associated with exacerbated malnutrition, retarded growth, decreased cognitive performance, and school absenteeism, and are therefore considered to be important obstacles to economic development [6].

Due to the health risks of poor sanitation, and their relevance to sustainable economic development, improvements to global sanitation are prioritized as a sub-

category of United Nations millennium development goal (MDG) #7: To ensure environmental sustainability. MDG #7 calls for a doubling of the proportion of the developing world's population having access to improved sanitation over the 1990 level of 36% by the end of year 2015. While significant progress has been made, with a 20% increase from 1990 to 2010 in the proportion of people in developing regions using improved sanitation, improvements must be accelerated significantly to achieve the stated goal of 72% coverage by the end of 2015 [7].

While the need for improved sanitation in developing countries is clear, there are numerous factors that complicate the implementation of sanitation improvements. One problem that has delayed the implementation of appropriate and sustainable sanitation in many areas is an overreliance on conventional piped sanitation schemes, which are usually prohibitively costly to implement [8], and are difficult to maintain even in wealthy countries such as the United States [9]. Many alternative approaches to sanitation are available, ranging from simple pit latrines to flush toilets connected with septic tanks. The common challenge to all approaches is how to safely dispose of excreta once it has accumulated. One strategy, which seeks to re-utilize excreta in horticultural applications after they are sanitized through composting or other processes, is known as ecological sanitation, or Ecosan.

Ecological sanitation systems are often viewed as a particularly interesting alternative strategy for resource-poor settings. Ecosan systems can, in theory, achieve safe disposal of excreta simultaneously to the production of saleable fertilizers and soil conditioners, making possible the subsidization of sanitation services through the sale of their treated output. Furthermore, most Ecosan treatment and reuse systems

require significantly less investment at startup, can be constructed and maintained using locally available materials, and require less specialized knowledge and infrastructure than conventional sewerage systems (for an informative review on the concept and practice of Ecosan see Ecological Sanitation by Winblad, Simpson-Herbert, and Calvert [10]).

In addition to the economic and logistical advantages of ecological sanitation, there are many environmental benefits to an Ecosan approach when compared to piped sanitation. These include conservation of water, containment and localization of hazardous fecal material away from waterways, beneficial application of nutrients present in human waste to terrestrial systems, reduced nutrient pollution of aquatic ecosystems, reduced chemical and energy demand for treatment of wastewater, and reduced risk of disease from water distribution system failures (a growing problem in the industrialized world [11]).

Health Risks and Standards for Safe Re-utilization of Ecosan Compost

Though Ecosan has many environmental and logistical benefits, it can present severe health risks unless all dangerous microorganisms present in excreta are inactivated prior to re-utilization. If incompletely sanitized materials are applied agriculturally, pathogens are likely to be provided with a direct fecal-oral route for infection. If unsafe materials are used in non-agricultural applications, workers and other individuals that interact with the site of re-use or disposal may be at elevated risk of infection. For these reasons, the WHO and the United States Environmental Protection Agency (USEPA) have set standards for the acceptable maximum pathogen content of recycled biosolids. USEPA requirements for Class A biosolids, which may be sold commercially without restrictions in use, include less than 3

Colony Forming Units (CFU) of *Salmonella* per 4 grams Total Solids (TS), less than 1 enteric virus per 4 grams TS, and less than 1 viable helminth ovum per 4 grams TS [12]. WHO standards require ecological sanitation systems to result in attributable estimated disease risks of less than 10^{-6} Disability-Adjusted Life Years (DALYs) per member of the population per year [13]. A less stringent standard of 10^{-4} DALYs per person per year has been proposed as more feasible, yet still likely to result in decreased incidence of diarrheal diseases over time [14].

Pathogens present in Ecosan excreta may be inactivated through a variety of processes. Sustained high temperatures (≥40º C) reached during thermophilic composting and solar heating are effective at destroying microorganisms. Alkalizing agents such as quicklime (CaO) can be used to raise the pH to a range (≥12) incompatible with microbial survival. The use of urine-diverting toilets, bulking agents, and storage under arid conditions can result in sufficiently low moisture (≤5%) to inactivate many microorganisms. In the simplest approach, long-term storage (on the order of years) may ensure that pathogenic microbes are inactive before excreta are reused. However, even after being treated, ecosan composts must be tested to ensure sufficient inactivation of key pathogens, especially helminth ova.

Viable helminth ova, particularly the eggs of *Ascaris lumbricoides*, are used as benchmark measures of pathogen inactivation and safety in sanitation systems, due both to their public health impact and to their persistence in the environment. *Ascaris* ova are the hardiest and longest-lived pathogens encountered in human excreta, surviving for more than 9 years under certain soil conditions [15]. The ova possess a complex, tri-layered protective structure that allows the embryos to resist environmental changes that are fatal to most organisms [16]. A sanitation system that

can be shown to inactivate the eggs of *A. lumbricoides* is likely to inactivate all other pathogens and render excreta safe for re-utilization. On the other hand, an ecological sanitation system that is ineffective at removing or inactivating *Ascaris* ova may place populations at high risk of morbidity, mortality, and developmental disadvantages associated with ascariasis (as observed by Corrales *et al.* [17]).

Ascariasis is the most common helminth infection worldwide, affecting an estimated 0.8-1.2 billion people, or 13-20% of the world's population in 2001. *Ascaris* is transmitted by ingestion of fully developed, larvate ova which mature in soil after being shed in feces. The ova adhere to and are ingested with soil particles and/or vegetation. Infections are concentrated mostly in Sub-Saharan Africa and Asia, but can be found throughout tropical and sub-tropical regions, especially where poverty and inadequate water and sanitation promote transmission of the parasites [3]. The highest intensity *Ascaris* infections tend to occur in children aged 5-15 years. Helminth infections tend to be concentrated heavily in a few 'wormy' individuals in communities where they are endemic, and aggregate by family and/or household [18-20].

In Bolivia, *Ascaris* prevalence and intensity varies with the climactic zone, from 1.4-8.9% in the altiplano (highland desert), to 5.0-83.0% in the temperate valleys, to 15.0-96.0% in the tropical zones [21]. A study by Flores *et al.* (2001) of 24 altiplano communities between La Paz and Lake Titicaca reported prevalences of *Ascaris* infection ranging from 1.2-28.0%, with the highest rates of infection recorded in male schoolchildren and children aged 5 to 8 years old [22]. While the cold, dry climate of the altiplano limits *Ascaris* transmission to some degree, ascariasis remains a public health concern in the region. Furthermore, Ecosan

fertilizers containing viable helminth ova originating in the altiplano may be sold and reused in warmer, wetter breadbasket regions where transmission potential may be higher.

Survival and Inactivation of *Ascaris* Ova in Soils and Compost

The life cycle of *Ascaris lumbricoides* is split between the human host and the external environment (Figure 1). During the external portion of the life cycle, shown in red, viable embryos develop, over the course several weeks, into infectious, larvate eggs. The fully developed ova must then be swallowed by a susceptible host for the life cycle to continue. Development of *Ascaris* eggs into their infective stages proceeds most quickly at temperatures between 20-32º C, in moist, shady environments, and aerobic conditions. Lack of oxygen can suspend parasite development, but the ova will continue to mature once aerobic conditions are restored [23].

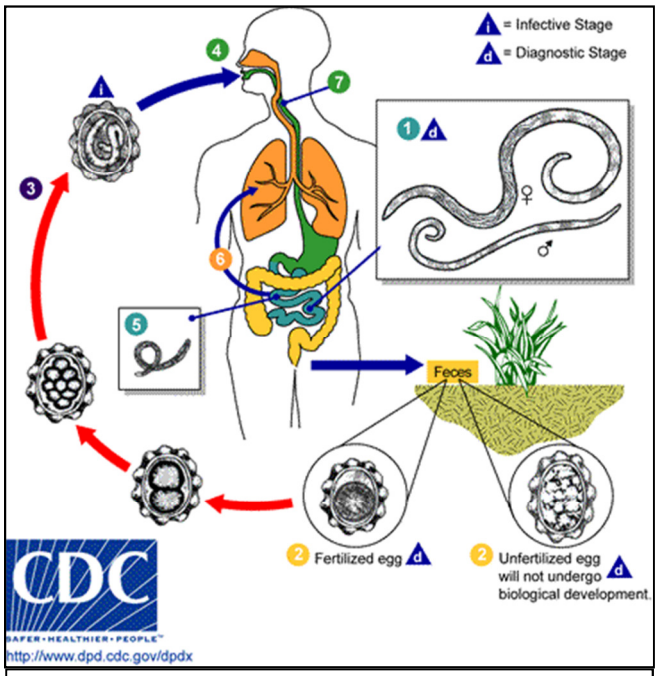


Figure 1: The Life Cycle of *A. lumbricoides*.
Source: *http://www.dpd.cdc.gov/dpdx/html/ascariasis.htm*

Though *Ascaris* ova are very robust to most environmental challenges, they can be destroyed or inactivated by a number of different processes. *Ascaris* ova are prey to certain decomposer fungi in soils [24]. High temperatures, alkaline treatments, exposure to ammonia, organic acids, drying, irradiation, aerobic and

anaerobic composting have been shown to inactivate *Ascaris* ova to some degree, and may be employed as treatment steps in sanitization of human waste, though with varying success in practice.

Inactivation through High Temperature Treatments

Exposure to elevated temperatures may be the most widely accepted means of inactivating *Ascaris* ova in compost. In a 1983 review of wastewater management, Feachem *et al.* state that, in the absence of specific ovicidal agents or prolonged storage, high temperature treatment is the only reliable way to achieve *Ascaris* egg elimination, claiming thermophilic aerobic composting as the most practical approach. Based on the results of several studies on the effect of elevated temperatures on *Ascaris* ova viability, Feachem *et al.* report that temperatures above 45-50º C promote rapid *Ascaris* inactivation [23]. Recent research continues to support these statements. In a 2003 prospective study, Moe and Izurieta found average peak temperatures at or above 36º C to be the single most important determinant of the rate of inactivation of *Ascaris* ova in solar toilets in rural El Salvador [25]. A 2007 study by Pecson *et al.* examined the effects of ammonia, pH, and temperature on the rate of Ascaris die-off in sewage sludge and found temperature to have the greatest impact [26]. A 2010 study by Maya *et al.* investigated the effects of 2-3 hour exposures of *Ascaris* ova in sludge to temperatures ranging from 30 to 80º C and observed 100% inactivation at temperatures at or above 70º C, 65-75% inactivation at 60º C, 25-35% inactivation at 50º C, 11-16% inactivation at 40º C, and very little inactivation below 40º C [27]. In another 2010 study, Hawksworth *et al.* examined the viability of *Ascaris* ova in

urine-diverting toilet sludge at a range of temperatures from 10ºC to 60º C, at 0% or 100% relative humidity, over a time span of 58 days. They found that at 60º C, near total inactivation was achieved within the first 24 hours regardless of relative humidity, while at 50º C, total die-off occurred after 32 hours at 0% relative humidity and after 96 hours at 100% relative humidity, and at 40º C, complete die-off occurred after 100 hours at 0% relative humidity and after 132 hours at 100% relative humidity. At temperatures below 40º C, ova remained viable through the end of the 58 day observation period regardless of relative humidity, though viability was consistently higher in 100% relative humidity samples [28].

Inactivation through Alkaline Treatments

Alkaline post-stabilization, a process in which a chemical such as quicklime is used to raise the pH of sludge to 12 or more, is frequently employed to treat biosolids due to its simplicity and low cost. This process has been shown to contribute to inactivation of *Ascaris* ova. However, alkaline post-stabilization appears incapable of inactivating helminth ova to < 1 viable ova/g dry weight in sludges with initially high ova concentrations without compromising agricultural potential and rendering disposal expensive and difficult [29].

A number of studies have investigated the effectiveness of alkaline treatments at eliminating *Ascaris*, with reported inactivation rates ranging from 94% within two hours (Mendez *et al.*) to 3.6% within one week of treatment (Plachy *et al.*) [30-37]. In the 2007 study by Pecson *et al.*, increasing pH from 7 to 12 was observed to accelerate *Ascaris* inactivation at temperatures of 30-40º C, though the effect of pH

was obscured by the effect of temperature at 50º C and was not significant at 20º C [26].

The heat generated by the exothermic hydration of quicklime (CaO) may account for some inactivation observed during alkaline post-stabilization. This effect is unique to quicklime and does not occur with other alkalizing agents, such as $Ca(OH)_2$ and ash, which may explain some disparities in the reported efficacy of alkaline stabilization [26].

Inactivation through Exposure to Ammonia

Conditions leading to the formation and retention of ammonia in compost have been shown to be effective at inactivating *Ascaris* ova. The formation and stabilization of uncharged ammonia (which readily crosses lipid membranes like those present in the protective shell of *Ascaris* ova) at high pH may be a major mechanism of pathogen inactivation during alkaline treatment, and differences in ammonia content may account for some contradictory reports on the efficacy of alkaline post-stabilization [26]. In addition to sanitization effects, ammonia stabilization has the added benefit of increasing the fertilization value of compost if the ammonia is retained as nitrogen.

In 2003, Vinneras *et al.* reported total inactivation of *Ascaris* ova in biosolids after 50 days of treatment with urea, which is converted to ammonia by the fecal urease enzyme. Ammonia concentrations in the treated waste were approximately 8000 ppm and were accomplished by adding urea concentrations roughly ten times as

high as those typical of a 50% urine/flush water mixture collected from Ecosan toilets [38].

A 2005 study by Pecson and Nelson was the first to explicitly examine the microbicidal effects of ammonia on *Ascaris* ova. In controlled laboratory solutions, they found that *Ascaris* inactivation was directly correlated to the concentration of uncharged ammonia, and that ammonia concentrations found in many sludges could reduce temperature requirements for 99% inactivation by up to 14º C (48º C for 99% inactivation in 72 hours without ammonia compared to 34º C for 99% inactivation in 72 hours with 8000 ppm NH_3 at pH 11) [39].

A 2009 study by Nordin *et al.* examined the effects of urea concentrations ranging from 0% to 2% (by weight) at 4, 14, 24, and 34º C on *Ascaris* ova viability in feces. At 34º C, ova in urea-amended feces were completely inactivated within 10 days, while in feces without added urea, ova took a month to be inactivated. At 24º C, only ova in 1% or 2% urea-amended feces, maintained at pH $\geq$ 10, were inactivated within one month. The authors concluded that ammonia is an efficient sanitizing agent at concentrations > 60 mM (~1,024 ppm), provided that compost temperatures are above 24º C. They posited that an ammonia concentration of ~280 mg/L would be the minimum threshold for eventual inactivation of ova [40].

Most recently, a 2012 study by McKinley *et al.* examined inactivation of *Ascaris* ova by ammonia released from urine and ash additives to ecological toilet composts. Inactivation of 99% of ova was achieved within 8 weeks when stored urine (in which some urea had decomposed to ammonia) and ash were added to the compost, but required 19 weeks when fresh urine and ash were added together. When fresh urine or ash was added separately to compost, inactivation was observed to

proceed after an 11-week lag phase, even though ammonia concentrations in the matrices were below the threshold of 280 ppm for *Ascaris* inactivation hypothesized by Nordin *et al.* This finding suggests that lower ammonia concentrations can be effective when combined with prolonged contact times [41].

Inactivation through Treatment with Organic Acids

Treatment processes using acidic additives have been shown to rapidly inactivate helminth ova. The chemical structures of many organic acids interfere with cellular processes and are able to disrupt the membranous barriers protecting helminth ova. Acetic and peracetic acid are particularly effective, although high doses may be necessary to compensate for reaction with organic material in the matrix [42]. Barrios *et al.* report that peracetic acid at 550 ppm is capable of reducing viable helminth ova in highly contaminated sludge (74-142 helminth ova/g total solids) by 99-99.9% within ten minutes [43]. On the other hand, Nordin *et al.* reported no cumulative inactivation of *Ascaris* ova by peracetic acid, though it should be noted they only tested very low doses [38] .

Inactivation through Drying

The moisture content of a compost or soil is an important factor for *Ascaris* survival. When the moisture content of a soil or compost holding *Ascaris* ova falls below 5 percent, inactivation may proceed rapidly [23]. In studies of high temperature inactivation of *Ascaris* ova, higher moisture content is invariably protective of the ova [27, 28]

Inactivation through Irradiation

Ascaris ova are susceptible to damage from irradiation, and survive longest below the surfaces of soils and composts [23]. However, relatively large doses of radiation are required to inactivate *Ascaris* ova, which are naturally protected by the outer layers of their shells. Brownell *et al.* (2006) report a 1.80 (+/- 0.32) log reduction in viability of decorticated ova (in which the outer layers of the shell have been removed) exposed to 500 J/m^2 254 nm UV fluence [44]. Intact ova exposed to the same fluence registered only a 0.44 (+/- 0.20) log reduction, suggesting that the outer protein coat and chitinous shell of *Ascaris* ova provide substantial protection from UV radiation.

Inactivation in Aerobic and Anaerobic Composting Systems

Aerobic and anaerobic composting processes can inactivate *Ascaris* through the release of heat or by the generation of toxic chemicals, such as acetic and peracetic acids and ammonia. Generally, aerobic processes release heat more efficiently, while anaerobic processes accumulate greater concentrations of organic acids and other toxic byproducts [45]. The sanitizing action of heat released during composting is greatly influenced by the extent to which a compost is insulated and well-mixed [46].

Koné *et al.* (2007) report reduction from 13-94 viable ova/g total solids to <1 viable ova/g total solids after 80 days of thermophilic aerobic co-composting of fecal sludge with organic market waste [47]. The system examined by Koné *et al.* maintained temperatures above 40º C for at least 20 days throughout the mass of the compost, which was most likely the critical mechanism accounting for the

inactivation of *Ascaris* ova. In contrast, a less successful aerobic composting system was investigated by Loc and Obertynska (2001), who reported insufficient pathogen reduction to meet regulatory standards after co-composting with sawdust at 20º C for 56 days [48]. The disparity between these results could be attributed mostly to the difference in temperature of the composts, and perhaps to the shorter treatment time in the less successful system.

Johnson *et al.* (1998) found more than 50% and 90% survival of unembryonated and embryonated *Ascaris* eggs after 5 weeks of mesophilic anaerobic composting at 35º C [49]. A similar result was reported by Juris *et al.* (1996), who found that only 17-18% of *Ascaris* eggs showed signs of damage after 20 days of anaerobic mesophilic digestion at 35º C [50]. From these two results, it would appear that anaerobic composting processes at temperatures below the thermophilic range are not particularly efficient at inactivating helminth ova.

Black *et al.* (1982) compared *Ascaris* ova inactivation in aerobic and anaerobic systems, reporting, after 15 days of treatment, 23% *Ascaris* egg destruction in an anaerobic digester held at 35º C and 38% egg destruction in an aerobic digester maintained at 20º C. They concluded that there is a temperature-independent advantage for aerobic digestion [51]. In contrast, Kato *et al.* found that *Ascaris* ova were more rapidly inactivated in anaerobic digesters than in aerobic digesters if temperatures were held constant for both processes. Most notably, when both digesters were held at 37º C, the anaerobic digester inactivated 75% of ova within 10 days, whereas the aerobic digester had no detectable effect on Ascaris viability [52].

The Suitability of Vermicomposting for Sanitization

One treatment strategy for Ecosan wastes that has received less attention in published research is vermicomposting, which uses the natural activities of earthworms to break down solid waste, converting it to humus-like material. As a step in the ecological sanitation process, vermicomposting has several appealing properties. Advantages of vermicomposting include rapid stabilization of biosolids accompanied by removal of odor and decreased attractiveness to flies, the release of bioavailable forms of important plant nutrients [53], aeration and mixing of the compost, and the sequestration of potentially toxic heavy metals (although the accumulation of heavy metals in the flesh of earthworms may present an ecological risk to species that prey on them [54]). Vermicomposting may also be more conceptually appealing to sanitation workers and users than other strategies, since decomposition of wastes by earthworms is a familiar natural phenomenon.

While vermicomposting has the capacity to improve productive potential of composted biosolids, there is less evidence on the ability of the process to inactivate human pathogens and produce compost that is safe for reuse in the absence of additional treatment. Notably, high temperatures, alkaline pH values, and low moisture contents known to be effective conditions for pathogen inactivation are not compatible with the conditions necessary to maintain earthworm populations, which require temperatures no higher than 35 degrees Celsius, a pH between 5 and 9 [55, 56], and greater than 50% moisture content by weight [57]. Alternative mechanisms by which pathogen inactivation might occur during vermicomposting include mechanical disruption in the worm gizzard, antimicrobial substances produced in the earthworm digestive tract, enzymatic digestion, and the stimulation of non-

pathogenic microbial competition in the compost [49]. However, the extent to which these phenomena affect survival of fecal pathogens is not well characterized. The following review summarizes published research relevant to the fate of bacterial, viral, and helminthic pathogens in vermicomposts.

Effects of Vermicomposting on Fecal Bacteria

A substantial number of studies have examined the fate of bacterial pathogens and indicator organisms during vermicomposting. Many studies report rapid and effective reductions in the bacterial populations, though a few report insignificant reduction, regrowth, or translocation of pathogens by composting worms.

At least five published studies have reported dramatic die-off of bacterial pathogens after vermicomposting. In 1976, M.J. Mitchell observed reductions in populations of *Salmonella enteritidis* and *E. coli* in biosolids composted with the earthworm *Eisenia fetida* relative to controls [56]. In 1981, Mitchell and Brown conducted a series of experiments examining the effect of *E fetida* on *Salmonella* survival which confirmed Mitchell's previous observations. Cultures grown for periods ranging from 4 to 28 days in the presence of *E. fetida* exhibited decreases in Salmonella populations of 97.8%-99.9% relative to cultures grown in the absence of the worm. Brown and Mitchell hypothesized that the earthworms were stimulating growth of competitive bacteria that were in turn eliminating most of the *Salmonella* in the cultures [58]. In 1991, Eastman *et al.* conducted a field study in which windrows of municipal biosolids were inoculated with fecal coliforms and *Salmonella*, among other pathogens. *E. fetida* was then added to one set of windrows while another set remained as controls, and samples were taken over the course of 7

days. The group found that by day 6, fecal coliform counts showed a 6 log reduction from initial levels in the vermicomposted rows, while the control rows showed less than a 2 log reduction. *Salmonella* populations were reduced by more than 8 logs in vermicomposted rows compared with a roughly 5 log reduction in the control rows [59]. In 1992, Murry and Hinckley observed a four-fold greater decrease in *Salmonella* inoculated onto culture plates containing horse manure when *E. fetida* was present compared to when it was absent (8% vs 2% after 48 hours, $p<0.05$) [60]. Recently, Yadav *et al.* (2010) reported reduction of total coliforms from 10^9 MPN/g to undetectable levels in *E. fetida* vermicomposts seeded with human feces. Unfortunately, no control composts were included for comparison [61].

At least five studies have found that the action of composting earthworms on pathogenic microbes is complex and may be selective in its effects. Haimi and Hihta (1987) found that vermicomposting of a mixture of wastewater biosolids and bark reduced the density of fecal coliforms by 40% after 7 weeks relative to controls, but appeared to increase the density of fecal streptococci [62]. Pedersen and Hedriksen (1993) observed reductions in the populations of *E coli* and *Pseudomonas putida* in the casts of earthworms that had been fed cattle dung, but noted no significant change in populations of *Enterobacter cloacae* and *Aeromonas hydrophila*. Interestingly, *E cloacae* concentrations were found to decrease by roughly a factor of four in the pharynx of the worms before subsequently re-growing further along in the worms' digestive tract until they approximated their initial values [63]. In a continuously-loading vermicomposting system fed with human feces, Buzie-Fru (2010) found significantly greater reductions in bacterial numbers in the presence of *E. fetida* than in control cultures for *E. coli* (3.74 log reduction vs. 0.26 log

reduction), *Enterococcus faecalis* (5.22 log reduction vs. 0.10 log increase), *Salmonella* (5.58 log reduction vs. 0.59 log reduction), *Enterobacter* (3.77 log reduction vs. 0.36 log reduction), and fecal coliforms (5.0 log reduction vs. 0.29 log reduction), but a statistically insignificant difference in inactivation for *Shigella* (2.50 log reduction vs. 1.54 log reduction) [64]. Aira *et al.* (2011) found that composting cow manure with *Eisinia andrei* resulted in reductions in the concentrations of fecal enterococci, fecal coliforms and *E. coli*, but did not appreciably reduce concentrations of *Enterobacteriae, Clostridium,* or total coliforms [65]. Recent research by Gomez-Brandon and colleagues (2011) on the structure of microbial communities formed in earthworm casts has shown that earthworm digestion exerts a bottleneck effect on microbial communities, reshaping different inputs from manures of various animals to biochemically indistinguishable outputs[66]. This finding strongly supports the idea that earthworm digestion may be selective, allowing certain types or species of bacteria to survive the process.

Early in the vermicomposting process, earthworms may actually distribute pathogens within material and facilitate their growth by bringing them to new environments. Williams *et al.* (2006) found evidence that epigeic (litter dwelling, horizontally traveling) earthworms can horizontally spread point contaminations of *E coli* O157:H7 in compost heaps and aid its proliferation in the short-term, though long-term survival was unaffected [67].

Taken together, these findings indicate that vermicomposting can effectively eliminate many bacterial pathogens, but some species may not be as vulnerable to its effects. However, if composting and storage times are sufficiently long, surviving bacterial pathogen populations would most likely dwindle as the availability of

biodegradable matter in the compost decreases and bacterial species specialized to soil environments out-compete pathogenic strains for resources. The ability of vermicomposts to rapidly stabilize biosolids would accelerate this endpoint relative to other forms of composting. However, in the absence of additional research to characterize the fate of specific bacterial pathogens in vermicompost, it should not be assumed that vermicomposting eliminates all pathogenic enteric bacteria.

Effects of Vermicomposting on Virus Populations

The fate of viruses during vermicomposting has received less study than that of bacteria. Eastman *et al.* (2001) seeded enteric viruses (species unspecified) into biosolids composted in the presence or absence of *E fetida*. After 6 days, they observed a 4.6 log reduction of enteric viruses in vermicomposted biosolids compared with a 1.8 log reduction in the control biosolids [59]. Lotzof *et al.* (2002) monitored the sanitary quality of vermicompost in a waste treatment facility that processed sewage by combining dewatered biosolids with shredded green wastes and subjecting the mixture to open-air vermicomposting by *E. fetida* for 60 days. According to the researchers, all the material tested at the facility, which was marketed as Grade A compost, contained less than one plaque forming unit per gram of compost [68].

Both these studies suggest that vermicomposting may be an effective means of eliminating viral pathogens from waste. Unlike bacteria, which are metabolically active in the environment and may have differing vulnerabilities to the process of vermicomposting due to differences in their metabolisms, human viruses are dormant in sewage, and can only be inactivated through physical or chemical destruction. It

seems likely that whatever processes in vermicomposting inactivate viruses would act in a nonspecific manner. Nonetheless, an important limitation to the results reported by Lotzof, Eastman, and their collaborators is their reliance on measuring virus counts by plaque formation on cell culture plates. One of the most important and common groups of human enteric viruses, the noroviruses, cannot be grown in culture by standard laboratory methods [69]. The presence of intact norovirus RNA can be measured by RT-PCR, although this technique does not measure infectivity [70]. While there may be no feasible means of measuring changes in norovirus infectivity in composts, it would be useful to assess changes in viral RNA concentrations of this important pathogen during the composting process in order to extend the results reported by Lotzof and Eastman.

Effects of Vermicomposting on Helminth Populations

A small number of studies have directly or indirectly examined the effects of earthworms or vermicomposting on helminths. The results of these studies are not always in agreement, and they include studies reporting effective inactivation of helminth ova in vermicomposts, studies reporting no observable effect or a protective effect of vermicomposting on helminth ova, and studies examining the hatching or transport of helminth ova within earthworms.

Studies Reporting Inactivation of Helminth Ova during Vermicomposting

Inactivation of helminth ova during vermicomposting has been reported in some studies, but in all cases it is unclear whether the vermicomposting process is the cause of the reduction in viability. Cardoso-Vigueros and Ramirez-Camperos

(2006) studied the effects of a compound process, involving thermophilic aerobic composting followed by vermicomposting, on the quality of sewage sludge and tannery waste products. They observed a decrease in the concentration of helminth ova from 3.3 ova/g <1 ova/gram. However, the role of the vermicomposting step in these reductions is doubtful. All composts were maintained above 50° C for several months during the thermophilic step, which would most likely have eliminated all pathogens before vermicomposting was performed [71]. Rodriguez-Canche *et al.* (2010) reported pathogen die-off in several experimentally constructed vermicomposting systems with 0, 1, 2, or 2.5 kg/m^2 *Eisenia fetida*. After 60 days of composting (which were preceded by 30 days of 'pre-composting' to make the material hospitable to the earthworms), viable helminth ova counts were reduced from 12.5/g in 'pre-composted' sludge to <1/g, regardless of earthworm density. While it appears that some factor in the overall pre-composting and composting process was sufficient to inactivate helminth ova, this reduction was observed even in the absence of earthworms, suggesting that vermicomposting was not the process responsible [72].

Another two studies, while not explicitly claiming to have observed reductions in helminth ova counts, are unintentionally misleading in reporting a finished vermicompost product that contained no detectable helminth ova. Vigueros and Ramirez Camperos (2002) and Contreras-Ramos *et al.* (2005) both report no detectable helminth ova in vermicomposted waste, but also observed no helminth ova in the waste prior to composting [73], [74]. These papers have been erroneously cited in the literature as supporting the ability of vermicomposting to inactivate helminth ova (e.g. [75])

Some research supports the idea that vermicomposting may be effective at destroying helminths that pass through a larval phase in soil, such as hookworm. Campos-Herrera *et al.* (2006) examined the fate of the entomopathogenic nematode *Steinernema feltiae* passing through the digestive system of *E. fetida* and found severely reduced mobility and infectivity in juvenile *S. feltiae* isolated from the earthworm castings [76]. Similar effects of earthworm predation on nematode populations outside the egg were observed by Dash *et al.* (1980) [77], which was cited as evidence that some enteroparasitic worms may be destroyed by the action of earthworms in the vermicomposting process [56]. These findings suggest that vermicomposting with *E. fetida* may have the capacity to inactivate helminths that pass through a larval stage in the soil before infecting humans, but should not be taken as evidence that vermicomposting destroys helminth ova, which are likely to be more resistant than the larvae they contain. Furthermore, Dash and colleagues reported variability by species in the effect of earthworm digestion on nematode viability, which emphasizes the need to confirm experimentally to what extent vermicomposting may impact the survival of hookworms.

Studies Contradicting Inactivation of Helminth Ova by Vermicomposting

At least two studies have found that the action of earthworms did not result in die-off of helminth ova. Ann E. Jones *et al.* (1979) tested the observable effects of unspecified earthworms on *Ascaris* ova present in sewage sludge spread over the surfaces of model pastures. They reported that ova recovered in the earthworm castings showed more advanced development than eggs recovered from the surface sludge. Furthermore, one week after the experiment had been initiated, less than 2%

of ova recovered from earthworm castings were observed to exhibit damage from fungal attack, while nearly 30% of ova recovered from the surface sludge exhibited signs of fungal infection. The authors assert that survival of the eggs of *Ascaris* and *Taenia* may be prolonged by passage through the earthworm digestive tract, which may protect the ova from fungal infection [78]. Bowman *et al.* (2006) examined viability and took gross counts of *Ascaris* ova recovered from potting soil to which *Eisenia foetida* had been added. They did not observe a statistically significant difference in viability in the presence or absence of *E foetida* after 1 week (93% and 91.8% viability, respectively). After 6 months, they observed a mean viability in ova recovered from the earthworm-treated material of 77.2%. Counts of all ova recovered showed no significant decrease over 183 days of treatment ($p=0.563$). The authors contend that this study shows that passage through the earthworm digestive system is not sufficient to inactivate or destroy Ascaris ova, though they concede that their experimental system was not a sewage-treatment vermicompost, and other mechanisms than digestion by earthworms may account for reports of Ascaris inactivation by vermicomposting in such matrices [79].

Studies Relating to Transport of Helminth Ova during Vermicomposting

Another set of studies which bears consideration investigates the tendencies of earthworms to transport helminths in the soil as paratenic hosts. Helminths may be transported as unhatched ova, or may be induced to hatch in the earthworm gut. In the latter case, hatched larvae may then be shed in worm castings, which would destroy any species that are not viable as larvae outside of their host, or may persist

in the tissues of the earthworm until it is consumed by a predator (as observed for the parasites *Toxocara catii* and *Toxocara canii* [80, 81]).

Several published studies support the idea that earthworms can transport helminths within soil. Lonc (1980) examined the tendency of earthworms to disperse helminth eggs in soil by directly inoculating specimens of *Lumbricus terrestris, E. foetida,* and *Allolobophora caliginosa* with ova of the tapeworm *Taenia saginata*, as well as by adding point contamination of *Taenia* eggs or segments to soil that specimens of the three earthworm species were living in. All three species of earthworm were observed to spread *Taenia* eggs vertically and horizontally within the soil [82]. Similar conclusions, albeit for free-living stages of entomopathogenic nematodes, were drawn by Campos Herrera *et al.* (2006) and Shapiro *et al.* (1995) [76, 83]. Given the demonstrated tendency of earthworms to disperse helminths and helminth eggs in soil, it seems reasonable to conclude that, whatever the capacity of vermicomposting to inactivate helminths, point contaminations of helminth ova in a compost pile will be spread as vermicomposting proceeds.

The tendency of *Ascaris* ova to hatch within earthworms has been examined in several studies, which are divided in reporting that hatching is or is not induced. Szelagiewicz-Czosnek (1972) found larva of *Ascaris suum* in *Lumbricus terrestris* collected from outdoor pig runs, and, after injecting *A. suum* eggs directly into the esophagus of *L. terrestris*, found hatched and viable larvae in the body cavity of the earthworms. The larvae were found to be infective to guinea pigs and were recovered from the lungs and livers of the animals after inoculation [84]. Smirnov (1975) performed a similar experiment, injecting *A. suum* eggs into the esophagus of *Eisenia foetida*. He recovered hatched larvae, and reported that the larvae grew and molted

inside of the earthworm intestine, remaining in the intestinal lumen or migrating to the body cavity. When inoculated earthworms were fed to pigs, Smirnov reported infections detectable 7-11 days sooner after exposure than normal. He suggested this accelerated course of disease to be due to hatching and development of the parasites within the earthworm intestine prior to infection of the pigs [85]. Similarly, Shumakovitch and Migatchyova (1976) recovered hatched larvae and produced infections in guinea pigs from *Lumbricus rubellus*, *Eisenia foetida*, and *Aporrectodea longa* that had ingested *A. suum* eggs [86].

In contrast, Ishii and Hashimoto (1963) fed embryonated eggs of *Ascaris suum* to *Pheretima spp* and *Eisenia spp*, and found no evidence that Ascaris larvae hatched in the tissues of the earthworms[87]. Jakovljevic (1975) failed to recover *A. suum* larvae from *Aporrectodea, Octolasium*, and *Lumbricus spp.* after ingestion or injection of matured ova into the earthworm digestive tract, finding instead that the majority of infective eggs were recovered in the worm castings [88]. Kraglund *et al.* (1998) failed to recover hatched *Ascaris* larvae from either exposed *A. longa* worms, or pigs that ate the exposed earthworms [89]. Roepstorff *et al.* (2002) found no evidence that matured *A. suum* eggs hatched when fed to *L. terrestris* earthworms and failed to detect any larvae when using three different recovery techniques. Moreover, no *Ascaris* larvae were recovered from the lungs or livers of pigs fed 25 exposed earthworms each, though unidentified, non-ascarid nematode larvae were recovered from both the earthworms and the pigs [90].

In summary, the published evidence on the fate of *Ascaris* and other helminths during vermicomposting is not conclusive in any respect. No study has persuasively demonstrated the inactivation or destruction of *Ascaris* ova by

vermicomposting. Some studies that reported final vermicomposts without helminth content are erroneously cited as evidence of inactivation in the literature despite the absence of helminthes prior to vermicomposting. Other studies have provided evidence that passage through the earthworm gut may not be destructive and may even be protective to the ova of *Ascaris*, but have not examined vermicomposting conditions that would be found in an ecological sanitation system. Studies investigating the tendency of *Ascaris* ova to hatch inside earthworms are evenly divided in their positive and negative findings. The one conclusion on which there does not appear to be disagreement is that some helminth eggs ingested by earthworms will be deposited in casts as the earthworms move through the vermicompost pile, leading to diffusion of point contaminations of helminth ova. Additional research with more consistent experimental design is needed to assess the suitability of vermicomposting as a sanitization strategy in ecological sanitation, especially with respect to helminth ova.

Quantitative Microbial Risk Assessment of Ecological Sanitation Systems

Beyond characterizing the ability of a sanitization strategy to render safe compost, it is important to evaluate the actual risks that may be posed by residual pathogens in eco-san products, and to identify key variables in the system that determine these risks. This is the role of Quantitative Microbial Risk Assessment (QMRA). Steps in the process of a QMRA include **hazard identification**, in which the investigator identifies and describes the infectious organism(s) of interest, **exposure assessment**, in which the investigator determines the magnitude of exposure to each hazard, **dose-response analysis**, in which the likelihood of

infection is assessed for a range of exposures, and **risk characterization**, in which the likelihood of infection and illness in a specific population is calculated given the likelihood and magnitude of exposures and the dose-response analysis [91].

Considerations specific to QMRAs of excreta reutilization in horticulture revolve mainly around the complex processes leading up to exposure. Most pathogens responsible for risks in excreta reutilization must be ingested to cause infection and illness, and so there must be a pathway by which pathogens present in reused excreta become attached to food crops, and survive on crops until consumption by a susceptible population. In contexts outside of consumption of food grown with Ecosan composts, risk pathways could include the transfer of pathogens present in reused excreta to the hands of workers or visitors to the site of reuse, and the transfer of pathogens from the contaminated hands to the mouth. In a shorter form, such a risk pathway may be expressed as the rate at which soil contaminated by the reused excreta is swallowed. The data used to estimate these key parameters should be as closely related as possible to the population of interest in the risk assessment, reflecting the characteristics of locally consumed crops, agricultural practices, and dietary habits.

In addition to the parameters of exposure estimation in waste reuse risk assessments, the dose-response relationship for *Ascaris* must be considered. There are no clinically derived dose-response data for *Ascaris*. Early attempts at risk assessment (e.g. [92]) for *Ascaris* infection have used a single-hit exponential model to estimate infection risks at dosage *d*:

$$P_i = 1 - e^{-d}$$

This model assumes that there are no protective factors against *Ascaris*

infection, and that infection is all but guaranteed with exposure to even a few viable ova. Navarro *et al.* (2009) developed a dose-response model for *Ascaris* ova based on differences in helminth ova content of wastewater used for irrigation and differences in infection rates among children younger than 15 years between two sites in the Mezquital valley, Mexico [93]. The authors found that the data best fit a beta-Poisson function:

$$P_i=1-(1+(d/859)(2^{1/0.104}-1))^{-0.104}$$

The model has since been utilized by Mara *et al.* (2010) [94], to calculate risks from wastewater reuse. However, the exact methodology with which Navarro *et al.* derived their model is unclear. Navarro's dose-response model may be less applicable to populations outside of the area of Mexico from which it was derived or for age groups other than children younger than 15 years.

Estimation of Burden of Disease from *Ascaris* Infections

In order to evaluate the suitability of a sanitation process that carries a risk of propagating infections, it is important to estimate the burden of disease such infections are likely to produce within the population. The estimated burden of disease for the system may then be compared to a WHO proposed maximum acceptable burden of 10^{-4} DALYs (Disability-Adjusted Life Years) per person per year [14, 94].

Models have been constructed by Chan *et al,* Bundy *et al*, and de Silva *et al* which estimate the burden of Ascariasis in communities based on the prevalence of *Ascaris* infection [5, 95, 96]. The method employed by these models is to estimate the prevalence in each age group based on the overall prevalence in the community, then to estimate the proportion of each age group with worm burdens above certain

thresholds for disease based on the prevalence of infection in the age group. DALYs lost to disability and death are then calculated according to modeled or assumed frequencies and severities of the effects of *Ascaris* infection in populations at risk.

Based on community prevalence, *s*, the prevalence in adults (15 years or older) is calculated from

$$P_{15+} = s / \sum_j (a_j * d_j)$$

where a_j is the prevalence weight for each age group and d_j is the proportion of the population in that age group (see **Table I** for age-dependent prevalence weights). The prevalence in each age group under 15 is calculated from

$$P_j = P_{15+} * a_j$$

Within each age group, the distribution of worm burdens is assumed to follow the negative binomial distribution with mean worm burden μ and species-specific aggregation parameter k ($k = 0.02$ for *Ascaris lumbricoides*, and is assumed not to vary by age group). These are related to the age-specific prevalence P_j:

Table I: Age-dependent Prevalence Weights

Age (years)	Prevalence Weight a_j
0-4	0.75
5-9	1.2
10-14	1.2
15+	1

$$P_j = 1 - \left(1 + \frac{\mu_j}{k}\right)^{-k}$$

The proportion of individuals, $p(x)$, with worm burden x is calculated from

$$p(x)_j = \left(1 + \frac{\mu_j}{k}\right)^{-k} * \frac{\Gamma(k + x)}{x! * \Gamma(k)} * \left(\frac{\mu_j}{\mu_j + k}\right)^x$$

Where $\Gamma(n)$ is the gamma function. The proportion of the of the age group at risk for morbidity, *Morb(P, T),* which is to say the proportion with worm burdens at or above

threshold *T* (see **Table II** for age-dependent threshold worm burdens), is then calculated from

$$Morb(P_j, T) = 1 - \sum_{x=0}^{x=T-1} p(x)_j$$

Table II: Age-dependent Lower and Higher Threshold Worm Burdens		
Age (Years)	**Lower Threshold Worm Burden**	**Higher Threshold Worm Burden**
0-4	7	15
5-9	15	30
10 and over	20	40

Once the proportion of the age group at risk of morbidity has been calculated, the burden of disease in Disability Adjusted Life Years (DALYs) may be estimated. DALYs lost to each outcome are calculated using the following equation:

$$DALYs = \int_{x=a}^{x=a+L} DW(x)e^{-0.03(x-a)}dx * Morb(P_j, T)$$

Where *a* is the age of onset, *L* is the duration of disability, (in the case of death or lifelong disability, L= 81.3 – *a*), *D* is the disability weight, *W(x)* is the age weight, $e^{-0.03(x-a)}$ is a factor discounting the value of future years by 3% per year, and $Morb(P_j, T)$ is the proportion of the age group at risk for the outcome in question. The burden of disease from each outcome across all age groups is calculated by adding together the products of the burden of disease for each age group and the proportion of the total population made up by that age group. The sum of the burdens of disease from each outcome may then be calculated to estimate the overall burden of disease attributable to *Ascaris* infection within the population.

In the case of *Ascaris* infection, the burden of disease is assumed to be made up of four outcomes:

Type A: Contemporaneous disability that occurs in individuals with worm burdens above the higher threshold and is recovered once the infection is cleared;

Type B: Permanent disability that occurs in a small proportion of children younger than 15 with worm burdens above the lower threshold;

Type C: Acute complications (intestinal obstruction, biliary Ascariasis) requiring hospitalization that occur in a small proportion of individuals with worm burdens above the higher threshold;

Type D: Mortality, which occurs in a small proportion of individuals experiencing acute complications;

These outcomes, the groups they affect, their duration, and disability weight are summarized in **Table III**.

Table III: Outcomes of *Ascaris* Infection Used in DALY Calculations				
Type	**Description**	**Groups Affected**	**Disability Weight**	**Average Duration by Age Group**
A	Temporary disability that is recovered once infection is lost	All individuals with worm burdens over the higher threshold	0.096	All ages: 1 year
B	Permanent developmental deficiencies that are not recovered after infection has been cleared	3% of Children <15 years old with worm burdens over the lower threshold	0.096	0-4 years: 81.3 years 5-14 years: 71.7 years
C	Acute complications (intestinal obstruction, biliary *Ascariasis*) requiring hospitalization. Risk calculation is based on observed rates of intestinal obstruction as a function of infection prevalence.	All individuals with worm burdens over the higher threshold are at risk. $Y = 0.\ 0.3184\ X^2$ calculates the annual incidence of complication per 1000 individuals as a function of the prevalence of *Ascaris* infection (X). The overall incidence of complication is then divided among age groups as follows 0-5 Years: 0.375 Y 5-10 Years: 0.375 Y 10-15 Years: 0.225 Y 15+ Years: 0.025 Y	0.400	All ages: 4 weeks (0.077 years)
D	Mortality associated with acute complications	5% of all individuals experiencing acute complications (Type C Morbidity)	1	0-4 years: 81.3 years 5-14 years: 71.7 years 15-44 years: 51.9 years 45-59 years: 32.5 years 60+ years: 14.9 years

Although this model has been used in peer reviewed literature to estimate the global burden of disease associated with *Ascaris* infection, it is not without defects. Bundy *et al.* note that the estimates of worm burden thresholds and disability weights may underestimate the burden of disease in children under 10. They also mention that the model does not quantify the effect of helminth infections as risk factors for other diseases, especially malnutrition.

Study Description and Context

This study was undertaken to examine the fate of *Ascaris* ova in a vermicomposting Ecological Sanitation system in El Alto, Bolivia, as well as to estimate health risks from viable *Ascaris* ova present in the vermicomposted excreta. Counts of viable and total *Ascaris* ova recovered from composts treated for 3 to 18 months are presented. Bayesian analysis is used to estimate the decay rate of *Ascaris* ova within the composts, and quantitative microbial risk assessment is applied to project the burden of disease from *Ascaris* infection that may result from the use of vermicomposted biosolids from this system to agricultural crops or public green space.

This research was performed in collaboration with the Bolivian NGO Sumaj Huasi, which, at the time of the study, provided ecological sanitation services to 687 households (the number has since grown) in the marginalized neighborhoods of San Roque and Villa Mercurio, on the northwestern outskirts of El Alto. The area's population is mainly comprised of recent immigrants to the city, many of whom live in houses that have only been partially constructed. There are no municipal sanitation or garbage services, and the area is highly polluted with garbage and human and animal excreta. Sumaj Huasi collaborates with residents to help them build urine-diverting ecological toilets on their properties, and provides excreta collection and disposal services.

The sanitation services provided by Sumaj Huasi consisted of bimonthly pickup of stored urine and feces (mixed with ~65% bulking materials such as sawdust and kitchen waste) from household toilets and delivery of the materials to one of two composting centers in San Roque or Villa Mercurio. The composting center at San Roque is a walled facility featuring several large plastic tanks for the storage of urine, a well for drawing water, four vermicomposting pits holding approximately 0.85 m^3 of compost each, and an experimental facility for testing the effects of the compost on plant growth. After collection, the material is seeded with

Figure 2: The Composting Center at San Roque

Eisenia fetida and maintained at 60-70% humidity through the addition of well water obtained on-site, as well as the use of a mesh cover to scatter radiation and retain moisture. Material is composted in the same container for the duration of its treatment.

The composting center at Villa Mercurio was still under development at the time of this study. There were no facilities for urine storage and no wall to prevent intrusion by animals. The composting pits at Villa Mercurio were larger and of irregular size. Composting material was gathered and prepared in a similar manner to the site at San Roque, but was moved from pit to pit depending on its age.

Figure 3: The Composting Center at Villa Mercurio

At both composting sites, the ambient environmental conditions were those of El Alto in general. The climate of El Alto, which is roughly 13,500 feet above sea level, is that of a high altitude desert. Average temperatures year-round hover near 7° C with large differences in temperature between sunlight and shade. Temperatures often fall below 0° C, especially at night and during the winter months (June-September). The area receives 23.74 inches of rainfall per year on average, with the majority of precipitation occurring between December and March.

Study Rationale

This study addresses important gaps in knowledge regarding the sanitization of biosolids and potential disease risks in ecological sanitation systems. The quantification of *Ascaris* ova and estimation of ova inactivation rates provide valuable data and evidence on the fate of helminth ova in vermicomposts. This issue has not been well-resolved in the scientific literature and is of growing importance as ecological sanitation gains prominence in international development projects. The quantitative microbial risk assessment provides estimates of the health risks from reuse of Ecosan excreta in several scenarios, and is one of very few risk assessments for *Ascaris* infection propagated by reuse of composted biosolids in horticulture.

In addition to the scientific needs addressed by the study, the practical information needs of Sumaj Huasi and the population they serve are also addressed. Sumaj Huasi operates on grants from the Swedish Development Agency (SIDA), but the long term sustainability of their sanitation services will depend on the possibility of marketing the processed urine and biosolids as fertilizer for horticultural use. The results of the QMRA performed in this study will inform policy recommendations to help Sumaj Huasi achieve safe reuse of the excreta they process, and will hopefully contribute to the financial sustainability of their sanitation services.

Study Goal and Aims

Goal: To assess the suitability of vermicomposting as the sole sanitizing process in an ecological sanitation system

Aim 1: To measure and report *Ascaris* content of vermicomposts processed for 3, 6, 8, 13, and 18 months in an ecological sanitation system in El Alto, Bolivia

Aim 2: To generate estimates of the rate of inactivation of *Ascaris* ova in the vermicomposts.

Aim 3: To construct and perform quantitative microbial risk assessments to estimate the burden of disease from *Ascaris* infection that would be attributable to the reuse of vermicompost from this system in horticultural applications including

(i) Risk assessment for consumption of raw carrots and spinach grown with vermicompost
(ii) Risk assessment for agricultural workers laboring on fields fertilized with vermicompost
(iii) Risk assessment for children aged 5-9 years playing in public parks where ground cover has been fertilized with vermicompost

Study Significance

The information and analyses in this study will contribute to the body of knowledge on sustainable sanitation interventions for low-resource settings, which are crucial for the prevention of millions of deaths and disease episodes each year and pertinent to economic development and environmental sustainability. The analyses from this project will directly benefit the providers and beneficiaries of an ecological sanitation system in El Alto, Bolivia, and help to ensure the safety and sustainability of that system.

Methods

Sampling

At the San Roque site, nine samples of roughly 100 g were drawn from each of three vermicomposting trenches, containing material that had been composting for 3, 8, or 13 months. At the Villa Mercurio site, three samples of roughly 100 g were drawn from each of two vermicomposting trenches, containing material that had been composting for 6 or 18 months. Samples were drawn from a wide spatial distribution within each pit, using a trowel to take material from the top, middle (roughly 20 cm deep), and bottom layers (roughly 40 cm deep). After collection, samples were transported to the laboratory at Universidad Mayor de San Andreas, Cota Cota, and stored at 4º C.

Recovery and Quantification of *Ascaris* Ova

Composite samples meant to represent all strata of the composts were created by combining 20 g of compost from the top, middle, and bottom layers of each sample. Each 60 g composite sample was then processed using a protocol adapted from the United States Environmental Protection Agency method for detection and viability determination of *Ascaris* ova in sludge [12]. To summarize, the samples were suspended in 200 mL of distilled water overnight at 4º C. On the following day, the samples were homogenized in a blender and stored overnight in 0.04% Linbro 7X at 4º C. Next, the samples were re-suspended in 0.1% Linbro 7X and filtered through a porosity #20 sieve in order to remove large particles. After being stored an additional 2 hours at 4º C, the samples were transferred to 50 ml conical tubes, with roughly 10 mL of sediment per tube. After addition of distilled water to a volume of 50 ml, the conical tubes were centrifuged at 1000 x g for 10 minutes, and the

supernatant was discarded. Magnesium sulfate solution with a specific gravity of 1.2 was added to each conical tube, and the sediment was resuspended, mixed, and centrifuged at 900 x g for 10 minutes. The supernatant, containing ova, was then poured through a porosity #400 sieve, retaining the ova on the top mesh. Using distilled water, the ova were rinsed into 15 mL conical tubes, and stored in 4 mL 0.1N sulfuric acid.

The 15 mL conical tubes containing the recovered ova were incubated at 28º C. Concurrent to the incubation of each sample, a positive incubation control was created by adding 5,000 *Ascaris suum* ova (Excelsior Sentinel Inc.) to 4 mL 0.1 N sulfuric acid, labeled with the date, and placed in the incubator. Samples and incubation controls were shaken daily for aeration, and their fluid volume was maintained by addition of distilled water. After 15 days of incubation, a small aliquot of the incubation control was placed on a microscope slide, and 100 ova were counted. If 90% of the ova counted in the positive control were embryonated to the infective stage, the sample was withdrawn from incubation and counted. If less than 90% of the eggs were embryonated to the infective stage, the incubation control was replaced in the incubator, and checked again after one to two days.

After 90% of the incubation control eggs had embryonated, samples were placed in a 40º C water bath for 3 minutes in order to induce movement in any larvae present. The total volume of each sample was recorded prior to counting, the sample was shaken to resuspend the ova, and 1.5 mL were transferred to a 1,000 square Sedgewick-Rafter slide. Using a light microscope, ova and larvae were counted in 100 grid squares, moving diagonally so as to include both central and peripheral grid squares in the count. The number of observed helminth larva and *Ascaris* ova were

recorded by square, and classified as unembryonated (no cell divisions evident, undefined mass in center of egg), embryonated (some development and cell division visible), embryonated with immature larva, or embryonated to the infective stage (fully developed larva visible inside of egg). Any movement of larvae was recorded. All ova embryonated to the infective stage were assumed to be viable.

Measurement of Physical Characteristics of the Compost Samples

Composite 20 g samples were created by adding together 6.67 grams from the top, middle, and bottom layers of each compost sample. Each 20 g composite sample was mixed into 20 mL of distilled water, and pH was measured using a pH electrode (Oakton® Acorn pH 6).

Percent humidity was measured for each sample by placing a plastic cup on a balance, and adding to the cup 10 g from each sample layer to give a total of 30 grams. Plastic cups loaded with sample were then placed in a large cardboard box covered in gauze, and stored at ambient temperature. Cups were re-weighed each consecutive day until their weights stopped changing, and the ratio of the total dry weight minus the weight of the plastic cup to the total original weight minus the weight of the plastic cup was used to calculate percent humidity.

Data Management

Data for each sample, including dates collected and processed, observations during processing, *Ascaris* counts, pH and humidity measurements were entered by hand onto standardized forms. These forms were then transcribed and double entered into an Excel spreadsheet for storage and analysis.

Bayesian Statistical Model for Ova Concentrations

In order to contend with the scarcity of data from the tested samples,

Bayesian hierarchical models were constructed estimating likely distributions of ova concentrations for each composting time. The Model was constructed in OpenBUGS (OpenBUGs Foundation) with the general form

$$\boldsymbol{ln(\alpha) \sim Normal(\mu_1, \tau_1)}$$

$$\boldsymbol{ln(\beta) \sim Normal(\mu_2, \tau_2)}$$

$$\boldsymbol{For\ (i\ in\ 1{:}N)}$$

$$\{\boldsymbol{Concentration[i] \sim Gamma\ (\alpha, \beta)}$$

$$\boldsymbol{Observed\ Ova\ Count[i]\ =\ Poisson(Mean\ Ova\ Count[i])}$$

$$\boldsymbol{Mean\ Ova\ Count[i]\ =\ Concentration[i] * Counted\ Mass[i]}\}$$

Where N is the number of observations for each composting time, *Concentration*[i] is the modeled mean concentration of ova in the sample, *Observed Ova Count*[i] is the number of ova counted in the sample, *Mean Ova Count*[i] is the modeled mean number of ova that would be counted in the effective mass examined for each sample, and *Counted Mass*[i] is the effective mass of each sample in which ova were counted. In the model, the number of ova counted in each sample is assumed to be drawn from a Poisson distribution with an expected value equal to the true mean number of ova that would be counted in a truly representative sample of the same effective mass. The hypothetical mean ova count is set equal to the product of the effective mass examined and the concentration of ova within the sample. The concentration of ova within each sample is drawn from a gamma distribution with shape parameter α and rate parameter β. The α and β parameters describe the variation between samples within each composting time, and are defined as

independent log normally distributed with μ and τ parameters. The τ parameters for α and β were varied for each composting time within the range of 0.1 to 1 to optimize Metropolis Acceptance Rates. A total of 100,000 concentration values were simulated for each composting time using Markov chain Monte Carlo simulations and the Metropolis-Hastings algorithm.

Bayesian Models for *Ascaris* Inactivation Rate in the Vermicomposts

Hierarchical Bayesian models were constructed in OpenBUGS to estimate the inactivation rate of *Ascaris* ova in the vermicomposts. In ***Model 1***, initial concentrations of viable ova in each sample were treated as unknown. In ***Model 2***, the number of ova counted in each sample that exhibited any visible development was assumed to represent the initial number of viable ova that would have been counted in that sample prior to vermicomposting, and was used to inform estimates of the rate of inactivation.

Model 1:

$$For\ (i\ in\ 1{:}N)$$

$$\{ln(Conc_{initial})\,[i] \sim Normal\ (\mu = Meaninitialconc_{site}, \tau_1)$$

$$ln(Conc_{true}[i]) = ln(Conc_{initial})\,[i] - Rate * T_{months}$$

$$Conc_{observed}[i] \sim Poisson(Conc_{true}[i])\}$$

$$For\ (i\ in\ 1{:}2)$$

$$\{Meaninitialconc_{site} \sim Normal\ (\mu_2, \tau_2)\}$$

$$\mu_2 \sim Normal(\mu_3, \tau_3)$$

$$\tau_1 \sim Gamma(\alpha_1, \beta_1)$$

$$\tau_2 \sim Gamma(\alpha_2, \beta_2)$$

$$ln(Rate) \sim Normal(\mu_4, \tau_4)$$

Where N is the number of samples taken at each composting site, $Conc_{initial}$ is the initial mean concentration of viable ova in the sample, $Meaninitialconc_{site}$ is the modeled mean initial concentration of viable ova across all composts at the composting site, $conc_{true}$ is the modeled mean concentration of viable ova in each sample at the time of observation, $Rate$ is the modeled rate of inactivation of viable ova per month, T is the composting time in months, and $Conc_{observed}$ is the concentration of ova in the portion of each sample that was counted. In the model, the mean initial concentration of viable ova in the composts at each site is assumed to fall within a normal distribution, the mean of which is also normally distributed, and the precision of which is gamma distributed. Within each site, the initial concentration of viable ova is assumed to fall within a log-normal distribution with a mean equal to the mean initial ova concentration for the site, and a precision that is gamma distributed. The observed concentration of viable ova within each sample is assumed to be drawn from a Poisson distribution with an expected value equal to the true mean concentration of viable ova in the sample. The log of the true mean concentration of viable ova in each sample at the time of observation is set equal to the log of the modeled initial mean concentration of ova minus the product of the modeled rate of inactivation of ova and the composting time of the sample. The rate of inactivation of *Ascaris* ova is assumed to fall within a log-normal distribution which is the same for all samples and all composting sites and is not assumed to be correlated with any other variables.

Model 2:

$$For\ (i\ in\ 1{:}N)$$

$$\{\ln(Conc_{initial})\,[i] \sim Normal\ (\mu = Meaninitialconc_{site}, \tau_1)$$

$$\ln(Conc_{true}[t,i]) = \ln(Conc_{initial})\,[i] - Rate * T_{months}[t,i]$$

$$Conc_{observed}[t,i] \sim Poisson(Conc_{true}[t,i])\}\}$$

$$For\ (i\ in\ 1{:}2)$$

$$\{Meaninitialconc_{site} \sim Normal\ (\mu_2, \tau_2)\}$$

$$\mu_2 \sim Normal(\mu_3, \tau_3)$$

$$\tau_1\ \sim Gamma(\alpha_1, \beta_1)$$

$$\tau_2 \sim Gamma(\alpha_2, \beta_2)$$

$$\ln(Rate) \sim Normal(\mu_4, \tau_4)$$

The variables in ***Model 2*** are the same as in ***Model 1***, and generally have the same relationships to each other as in the first model. However, in ***Model 2*** the linear equation relating the concentration of viable ova at the time of observation to the initial concentration and the decay rate is calculated for T=0 months and for T=t, the number of months spent composting at the time of observation. The Poisson distribution relating the observed concentration of viable ova in each sample to the true concentration of viable ova in the sample is also calculated for both T=0 and T=t. At T=0 months, the observed concentration of viable ova for each sample is assumed to have been equal to the observed concentration of ova exhibiting signs of development in the sample at the time of observation. In ***Model 2***, the estimation of the rate of decay is less uncertain, being informed by the assumption that any ova that were viable at the initiation of composting would remain in the compost and would have undergone a noticeable amount of development.

Microbial Risk Assessment Models

The risk to individuals of becoming infected with *Ascaris* was assessed for three different exposure scenarios: 1). Consumption of raw produce fertilized with vermicompost, 2). Accidental ingestion of soil by agricultural workers laboring on fields fertilized with vermicompost, and 3). Ingestion of soil by children playing in public parks where ground cover has been fertilized with vermicompost. Distributions of daily risks were constructed from 100,000 member Monte Carlo simulations. Annual risks were calculated by sampling daily risk distributions, and distributions of annual risks were constructed from Monte Carlo simulations repeating the calculation of annual risks. The models are shown on the following pages, with variables defined in **Tables IV, V, and VI**.

Model 1: *Ascaris* Infection Risk from Consumption of Raw Produce Fertilized with Vermicompost

$$Pinf_i = DoseResp(Consumption_{RV} * Wash * TSProp_C * Survival(Conc_{OM} * Density_{VC} * Application\ Rate * MixRatio * Transfer_{SAC}, T_{PC}))$$

$$Pinf_y = 1 - \prod_{i=1}^{Days_{prod}} (1 - Pinf_i)$$

Table IV: Model Parameters For Consumption of Raw Produce

Parameter	Description	Values	Sources	Key Assumptions
$Conc_{OM}$	Viable ova/g compost	Compost (99% CI) 3 Month ($1.23*10^{-5}$, 2.86) 6 Month ($2.50*10^{-6}$, 4.72) 8 Month ($1.33*10^{-3}$, 11.43) 13 Month ($1.22*10^{-5}$, 3.56) 18 Month ($1.21*10^{-12}$, 7.14)	Estimated from Bayesian models for ova concentrations in each compost	Modeled ova concentrations accurately predict real ova concentrations
$Density_{VC}$	The density of vermicompost Units: g compost/L compost	Distribution (99% CI) Norm(μ = (denslow + denshi)/2, σ = (denshi – denslow)/4) (133, 256) Where denslow = 168 g/L denshi = (244 g TS[1] /L) / propTS propTS ~ Norm (μ=0.277, σ=0.031)	Low density estimate obtained from Visvanathan *et al.*[97] High density estimate obtained from Mota *et al.* [98] Proportion of total solids in vermicompost was estimated from data collected in this study.	High and low estimates obtained from literature can act as 5th and 95th percentiles of a normal distribution, from which the density of vermicomposts in general can be estimated.

[1] TS – Total Solids. propTS designates the proportion of the mass of the vermicompost that remains after dehydration.

Table IV: Model Parameters For Consumption of Raw Produce (Continued)				
Application Rate	The volume of compost applied per unit area (L/cm^2)	Value 0.005 L/cm^2	Personal communication with Sumaj Huasi[99]	A point value was given, so no variability is modeled.
MixRatio	The dilution coefficient of compost at soil surface as it is mixed into soil	Values Conservative: 1.0 Less Conservative: 0.5 Least Conservative: 0.1	Assumed.	Assumes 100% of surface soil is compost, or it is mixed to 50% or 10% at the surface.
$Transfer_{SAC}$	Coefficient describing transfer of ova from soil to crops Unit Conversion: Ova/cm^2 soil → Ova/g total solids crop	Values For Spinach: 2.435 For Carrots: 0.591	Derived from Jimenez *et al.* [100]. Functions from points in publication were normalized to have y intercepts of 0 ova / g crop expected for soil concentrations of 0 ova /cm^2.	Implicitly assumes growing process identical to that of Jimenez *et al.* No helminth ova are assumed to be in soil prior to application of compost
Survival(x,t)	Function describing survival of *Ascaris* on crops after *t* days or as a proportion of original inoculum	Function *Conservative* Survival(x,t) = $x/(10^{t/t90})$ t90 ~ Norm (μ = 625 days, σ = 150 days) *Less Conservative* Survival(x) = SProp*x For Spinach: Sprop ~ Unif(0.50, 0.75) For Carrots: SProp ~ Unif(0.20, 0.25)	Conservative function taken from Schönning *et al.*[101] Less conservative function based on proportions of ova remaining viable as reported by Jimenez *et al.*[100] and Keraita *et al.*[102]	Conservative function assumes ova decay at same rate on crops as in soil. Less conservative functions are derived from point observations. Assume spinach is analogous to lettuce for *Ascaris* survival.
T_{PC}	Time (days) between planting and consumption of crops	Values: For Spinach: 56 days For Carrots: 91 days	Based on time to harvest reported by Jimenez *et al.* [100]	Crops are consumed 7 days after harvest.
$SProp_C$	Proportion of total solids in crops (Total solids/g bulk mass)	Values: For Spinach: 0.086 For Carrots:~Norm(μ=0.1171, σ=0.00429)	Data retrieved from USDA Nutrient Database [103]	

Table IV: Model Parameters For Consumption of Raw Produce (Continued)				
Wash	Coefficient describing the effect of a wash under running tap water to remove helminth ova from produce prior to sale	Value/Distribution Conservative models: 1 Less conservative models: ~Norm (μ=0.8, σ=0.3)/ ~Norm (μ=8.8, σ=1.55) If *Wash* < 0, set *Wash* = 0	Conservative value assumes no washing of produce Less conservative distribution based on information presented by Amoah *et al*. [104]	
$Consumption_{RV}$	Distribution describing raw vegetable consumption among Bolivians Units: g/day	Distribution (*99% CI*) ~Norm(μ=41.25, σ=33.25) (*0, 118*) If $Consumption_{RV} < 0$ Set $Consumption_{RV} = 0$	Based on daily vegetable consumption distributions for Urban Bolivians taken from Perez-Cueto *et al.* [105] and modified to avoid values < 0 g consumed per day	¼ of vegetable consumption is assumed to consist of raw produce, and in their respective models, either carrots or spinach are assumed to constitute 100% of this portion.
DoseResp Model	Dose-Response curve giving probability of infection as a function of number of viable ova ingested (D)	Exponential Function $P_{inf} = 1-e^{-D}$ Beta-Poisson Function $P_{inf} = 1-\left(1+\left(\left(\frac{D}{859}\right)*\left(2^{\frac{1}{0.104}}-1\right)\right)\right)^{-0.104}$	Exponential function is a traditional conservative assumption (e.g. [92]) Beta-Poisson function taken from Navarro *et al.*[93]	Exponential function assumes ingestion of a single ovum gives 100% risk of infection and exposure is Poisson distributed. Beta-Poisson function estimated from epidemiological data in children aged 5-15 in Mezquital valley, Mexico. Assumptions behind the model are not entirely clear. May not be accurate for other ages or outside the Mezquital valley.
$Days_{prod}$	Number of days each year in which consumers may ingest produce grown with vermicompost.	Conservative Value 365 Less Conservative Value 56	Assumed	Conservative assumption: produce is available year-round. Less conservative assumption: produce available for one month periods after each of two growing seasons.

Model 2: *Ascaris* Infection Risk from Ingestion of Soil by Agricultural Workers on Fields Fertilized with Vermicompost

$$Pinf_i(t) = DoseResp(Ingestion_{Farmer} * Survival(Conc_{OM} * Mixratio, t * 7))$$

$$Pinf_y = 1 - \prod_{j=0}^{19} for\ k\ in\ 1{:}6\ (1 - Pinf_i(j)_k)$$

Table V: Model Parameters For Ingestion of Soil by Workers on Site				
Parameter	**Description**	**Values**	**Sources**	**Key Assumptions**
$Conc_{OM}$	Viable ova/g compost	Compost (99% CI) 3 Month ($1.23*10^{-5}$, 2.86) 6 Month ($2.50*10^{-6}$, 4.72) 8 Month ($1.33*10^{-3}$, 11.43) 13 Month ($1.22*10^{-5}$, 3.56) 18 Month ($1.21*10^{-12}$, 7.14)	Estimated from Bayesian models for ova concentrations in each compost	Modeled ova concentrations accurately predict real ova concentrations
MixRatio	The dilution coefficient of compost at soil surface as it is mixed into soil	Values Conservative: 1.0 Less Conservative: 0.5 Least Conservative: 0.1	Assumed.	Assumes 100% of surface soil is compost, or it is mixed to 50% or 10% at the surface.
Survival(x,t)	Function describing *Ascaris* survival in soil	Function Survival(x,t) = $x/(10^{t/t90})$ t90 ~ Norm (μ = 625 days, σ = 150 days)	Taken from Schönning *et al.*[101]	Assumes ova decay at same rate as in type of soil Schönning's distribution describes (not stated).
t *k in 1:6* *j in* $Pinf_y$	Weeks since last application of compost on site. Used to calculate surviving concentration of ova.	Values: t, j: From 0 to 19 twice yearly k: from 1:6 for each iteration of t/j	Assumed	Compost is applied once in each of two growing seasons per year. Workers labor 3 days per week for 20 weeks during each growing season.
$Ingestion_{Farmer}$	Daily rate of soil ingestion for adult agricultural workers (grams/day)	Distribution: Unif(0.01,0.1)	Based on estimates used by Mara *et al.* [106] for ingestion of soil by workers in non-mechanized agriculture.	Assumes exposed workers engaged in non-mechanized labor. Assumes equal amounts of soil are ingested each day regardless of activities.

Table V: Model Parameters For Ingestion of Soil by Workers on Site (Continued)				
DoseResp	Dose-Response curve giving probability of infection as a function of number of viable ova ingested (D)	Exponential Function $P_{inf} = 1-e^{-D}$ Beta-Poisson Function $P_{inf} = 1-\left(1+\left(\left(\frac{D}{859}\right)*\left(2^{\frac{1}{0.104}}-1\right)\right)\right)^{-0.104}$	Exponential function is a traditional conservative assumption (e.g. [92]) Beta-Poisson function taken from Navarro *et al.*[93]	Exponential function assumes ingestion of a single ovum gives 100% risk of infection and exposure is Poisson distributed. Beta-Poisson function estimated from epidemiological data in children aged 5-15 in Mezquital valley, Mexico. Assumptions behind the model are not entirely clear. May not be accurate for other ages or outside the Mezquital valley.

Model 3: *Ascaris* Infection Risks from Ingestion of Soil by Children Playing in Parks Fertilized with Vermicompost

$$Pinf_i(t) =$$

$$DoseResp(Ingestion_{Child} * Wakingratio_{Park} * Survival(Conc_{OM} * Mixratio, t * 7))$$

For children living nearby:

$$Pinf_y = 1 - \prod_{j=0}^{12} for\ k\ in\ 1{:}12\ (1 - Pinf_i(j)_k)$$

For Children living at greater distance from the park:

$$Pinf_y = 1 - \prod_{j=1}^{3} for\ k\ in\ 1{:}Visits_{month}\ (1 - Pinf_i(j * 4 - 2)_k)$$

Table VI: Model Parameters For Ingestion of Soil by Children Visiting Parks

Parameter	Description	Values	Sources	Key Assumptions
$Conc_{OM}$	Viable ova/g compost	Compost (99% CI) 3 Month (*1.23*10^{-5}, 2.86*) 6 Month (*2.50*10^{-6}, 4.72*) 8 Month (*1.33*10^{-3}, 11.43*) 13 Month (*1.22*10^{-5}, 3.56*) 18 Month (*1.21*10^{-12}, 7.14*)	Estimated from Bayesian models for ova concentrations in each compost	Modeled ova concentrations accurately predict real ova concentrations
MixRatio	The dilution coefficient of compost at soil surface as it is mixed into soil	Values Conservative: 1.0 Less Conservative: 0.5 Least Conservative: 0.1	Assumed.	Assumes 100% of surface soil is compost, or it is mixed to 50% or 10% at the surface.

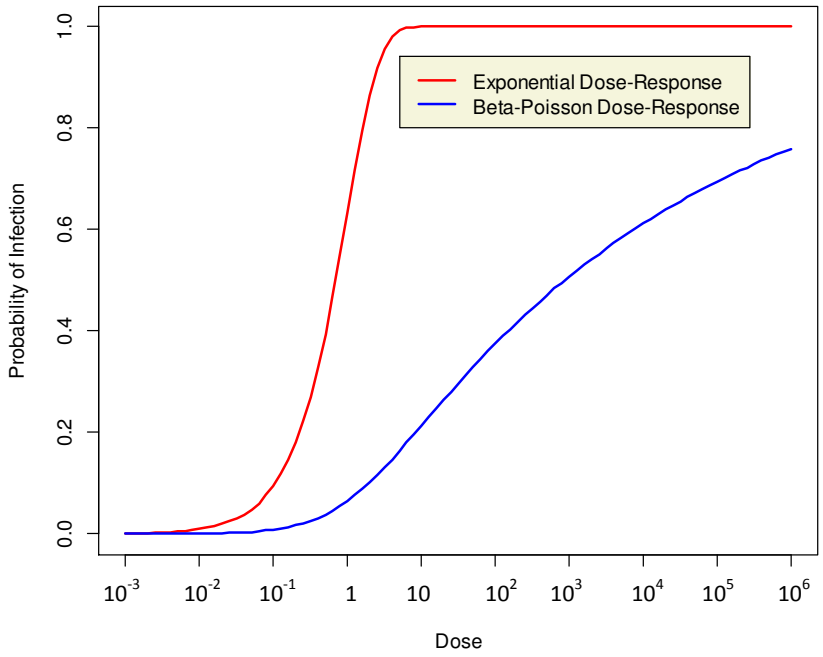
Table VI: Model Parameters For Ingestion of Soil by Children Visiting Parks (Continued)				
Survival(x,t)	Function describing *Ascaris* survival in soil	Function Survival(x,t) = $x/(10^{t/t90})$ t90 ~ Norm (μ = 625 days, σ = 150 days)	Taken from Schönning *et al.*[101]	Function assumes ova decay in local soil at same rate modeled for the type of soil Schönning's distribution describes (not stated).
t *j in $Pinf_y$ for nearby children* *j*4-2 in $Pinf_y$ for distant children*	Number of weeks since last application of compost. Used to calculate surviving concentration of ova for each week, or for distant children, month of exposure.	Values: For nearby children From 0 to 12 weeks 4 times yearly For distant children 2 weeks, 6 weeks, 10 weeks 4 times yearly	Based on personal communication with Dr. Juan Leon recounting his experience of the habits of children visiting parks in Bolivia [107]	Compost is applied to grass once in each of four growing seasons per year. Children living nearby visit parks 3 days per week year round. Children living at greater distance visit parks from 0-4 times per month.
$Wakingratio_{Park}$	The proportion of a child's waking hours spent at the park for each visit.	Distribution (*99% CI*) Norm(μ=1,σ=0.5)/16 (*0.15625, 0.135*) If $Wakingratio_{park} < 0.015625$ Set $Wakingratio_{park} = 0.015625$	Based on personal communication with Dr. Juan Leon recounting his experience of the habits of children visiting parks in Bolivia [107]	Assumes children are awake 16 hours each day, spend mean of 1 hour each visit, 95th percentile of 2 hours and minimum of 15 minutes. Soil ingestion rate does not vary.
$Ingestion_{Dhild}$	The daily rate of soil ingestion for children (grams/day)	Distribution: Ln Norm(μ=3.575, σ=1.193) 99% CI: (2.22, 576)	Taken from discussion section of Özkaynak *et al.* Created for 3-6 year olds. [108]	Assumes model suggested by Özkaynak *et al* translates for Bolivian children aged 5-9 years.
DoseResp	Dose-Response curve giving probability of infection as a function of number of viable ova ingested (D)	Exponential Function $P_{inf} = 1-e^{-D}$ Beta-Poisson Function $P_{inf} = 1-\left(1+\left(\left(\frac{D}{859}\right)*\left(2^{\frac{1}{0.104}}-1\right)\right)\right)^{-0.104}$	Exponential function is a traditional conservative assumption (e.g. [92]) Beta-Poisson function taken from Navarro *et al.*[93]	Exponential function assumes ingestion of single ovum gives 100% risk of infection and exposure is Poisson distributed. Beta-Poisson function estimated from epidemiological data in children aged 5-15 in Mezquital valley, Mexico. Assumptions behind the model are not entirely clear. May not be accurate for other ages or outside the Mezquital valley.

Table VI: Model Parameters For Ingestion of Soil by Children Visiting Parks (Continued)				
$Visits_{Month}$	Distribution describing the number of samples to draw for each simulated month of park visits for children living at a distance from the park	Distribution Ln Norm(μ=0, σ=0.693) If $Visits_{Month} > 4$ Set $Visits_{Month} = 4$ Round all values to nearest integer	Based on personal communication with Dr. Juan Leon recounting his experience of the habits of children visiting parks in Bolivia [107]	Assumes that children living at a distance from parks visit between 0 and 4 times per month, with a mean of 1 visit per month, and that the frequency of visitation can be thought of as being log normal distributed

Sensitivity Analyses

The effects of variable parameters on exposures and annual risk distributions were assessed for each model. Where possible, analyses focused on exposure, since risk rises quickly to 100% and increases in exposure may not be reflected in increased risk when using the exponential dose-response model (see **Figure 4** for a plot of the dose-response models). For the produce consumption models, the effects of viable ova concentration in the vermicompost, the dilution of compost in soil, the choice of function describing survival of ova on produce, a tap water rinse before market, the choice of dose-response function, and the number of days at risk were analyzed. For the models of ingestion of contaminated soil by agricultural workers and by children, the effects of the dilution of compost in soil and the dose-response function were analyzed. After arriving at plausible worst and best-case models, the effect of varying ova concentrations in compost was tested for each scenario to determine maximum allowable ova concentrations for an acceptable burden of disease.

Figure 4: Risk as a Function of Exposure for the Exponential and Beta-Poisson Dose-Response Models



Burden of Disease Calculations

In order to translate infection risks into an estimated burden of disease for comparison with the WHO proposed cutoff of 10^{-4} DALYs per person per year [14], it was assumed that incidence of infection could be set equal to prevalence (which might be expected after *Ascaris* transmission from the system becomes endemic to affected areas) and that the relationships modeled between prevalence and the distribution of worm burdens and infection among age groups by Chan, Bundy *et al* [95, 96] would hold true under the modeled scenarios. The burden of disease was then modeled for each scenario according to equations and values taken from Bundy *et al*:

$$P_{15+} = \frac{s}{\sum_j (a_j * d_j)}$$

$$P_j = P_{15+} * a_j$$

$$P_j = 1 - \left(1 + \frac{\mu_j}{k}\right)^{-k}$$

$$p(x)_j = \left(1 + \frac{\mu_j}{k}\right)^{-k} * \frac{\Gamma(k+x)}{x! * \Gamma(k)} * \left(\frac{\mu_j}{\mu_j + k}\right)^x$$

$$Morb(P_j, T) = \left(1 - \sum_{x=0}^{x=T-1} p(x)_j\right)$$

$$DALYs_{j,d} = \int_{x=a}^{x=a+L} DW(x) e^{-0.03(x-a)} dx * Morb(P_j, T)$$

$$DALYs_{allages,morbidity} = \sum_{j=0\ to\ 4}^{j=60+} DALYs_{j,d} * d_j$$

$$DALYs_{total} = \sum_{morbidity=type\ A}^{morbidity=type\ D} DALYs_{allages,morbidity}$$

Table VII: Variables in Burden of Disease Models		
Variable	**Description**	**Value**
P	Age-specific prevalence of infection	Calculated
s	Community prevalence of infection	Equal to annual infection risk from microbial risk models
a_j	Age-dependent prevalence weight (relative chance of infection by age group)	0-4 years: 0.75 5-9 years: 1.2 10-14 years: 1.2 15+ years: 1
d_j	Proportion of population at risk that is in the prevalence age group j	From 2008 Bolivia DHS [109] 0-4 years: 0.12 5-9 years: 0.135 10-14 years: 0.134 15-44 years: 0.408 45-59 years: 0.117 60+ years: 0.086
μ_j	Mean worm burden for age group	Calculated
k	Aggregation parameter (describes uneven distribution of infections in population)	0.02
$p(x)_j$	Proportion of individuals with worm burden x in age group j	Calculated
$Morb(P_j, T)$	Proportion of individuals with burdens above threshold T in age group j	Calculated
T	Age-dependent threshold worm burden for disease. Lower threshold entails milder morbidity, higher threshold severe morbidity	0-4 years Low: 7 worms, High: 15 worms 5-9 years Low: 15 worms, High: 30 worms 10+ years Low: 20 worms, High 40 worms
D	Disability weight for each outcome	Type A (temporary disability): 0.096 Type B (permanent developmental deficit): 0.096 Type C (complication requiring hospitalization): 0.400 Type D (mortality): 1
a	Average age of onset of disease for each age group	0-4 years: 2 years old 5-14 years: 10 years old 15-44 years: 30 years old 45-59 years: 50 years old 60+ years: 70 years old
L	Duration of disability	Type A Morbidity: 1 year Type B Morbidity: 81.3 or 71.7 years (by age group) Type C Morbidity: 0.077 years Type D (Mortality): 81.3, 71.7, 51.9, 32.5, 14.9 years (by age group)
$W(x)$	Age weight in DALY calculations	$0.16243xe^{-0.04x}$ [110]
$e^{-0.03(x-a)}$	Discounting factor at 3% per year into the future	Calculated

Table III: Outcomes of *Ascaris* Infection Used in DALY Calculations (Repeated from Literature Review)				
Type	**Description**	**Groups Affected**	**Disability Weight**	**Average Duration by Age Group**
A	Temporary disability that is recovered once infection is lost	All individuals with worm burdens over the higher threshold	0.096	All ages: 1 year
B	Permanent developmental deficiencies	3% of Children <15 years old with burdens over the lower threshold	0.096	0-4 years: 81.3 years 5-14 years: 71.7 years
C	Acute complications (intestinal obstruction, biliary *Ascariasis*) requiring hospitalization. Risk calculation is based on observed rates of intestinal obstruction as a function of infection prevalence.	All individuals are at risk. $Y = 0.3184\ X^2$ Where Y is the annual incidence of complication per 1000 individuals and X is infection prevalence. Overall incidence of complication is divided among age groups as follows. 0-5 Years: 0.375 Y 5-10 Years: 0.375 Y 10-15 Years: 0.225 Y 15+ Years: 0.025 Y	0.400	All ages: 4 weeks (0.077 years)
D	Mortality associated with acute complications	5% of all individuals experiencing acute complications (Type C Morbidity)	1	0-4 years: 81.3 years 5-14 years: 71.7 years 15-44 years: 51.9 years 45-59 years: 32.5 years 60+ years: 14.9 years

For the scenario of consumption of raw carrots and spinach fertilized with vermicompost, all age groups were assumed to be at risk. For the scenario of ingestion of soil by agricultural workers laboring on fields fertilized with vermicompost, individuals between 15 and 59 years old were assumed to be at risk. For the scenario of ingestion of soil by children playing in public parks with ground cover fertilized with vermicompost, children aged 5-9 were assumed to be at risk. For the latter two scenarios, the annual incidence of *Ascaris* infection calculated from the microbial risk models was applied directly as the prevalence for the population at

risk, omitting from the calculations those age groups not assumed to be at risk in the models.

Modeled Accumulation of *Ascaris* Ova in Soil with Repeated Applications

Due to the persistence of *Ascaris* ova in soil, repeated applications of compost containing viable *Ascaris* ova to the same area of soil may result in gradual accumulation of viable ova in that soil. The following model was constructed to estimate the number of viable ova persisting after each application when biosolids are applied every two years, once every year, twice per year, or four times per year:

$$Accumulation[T] = For\ (\ i\ in\ 1{:}T)\{\sum_{j=1}^{i} \frac{conc}{10^{\frac{(j-1)*timecoeff}{t_{90}\sim Norm(625,150)}}}$$

$$Monte\ Carlo\ Simulated\ 10,000\ Times\ for\ Each\ value\ of\ i$$

Where *T* is the number of applications to be modeled, *conc* is the value or distribution of values for concentration of viable *Ascaris* ova in the material being applied, and *timecoeff* is a coefficient converting the number of applications modeled (*T*) to a number of days (i.e. if a twice yearly application of compost is modeled, *timecoeff* = 182.5 days/application).

Results

Laboratory Measurements

Ascaris ova were detected in 27 of 31 samples. Viability ranged from 0-100%, with a mean value across all samples of 48%. Viable ova concentrations per gram vermicompost were highly variable, with most values clustering near zero viable ova/g total solids (TS), but some samples measured as high as 33 viable ova/g TS. The greatest numbers of viable ova were observed in the 8-month old vermicompost from San Roque. Generally, no hatching or vigorous movement of larvae inside the egg was observed in the samples or in the positive controls, with the exception of a single larva observed moving inside its egg in the 18-month old vermicompost from Villa Mercurio. Sluggish movement of larvae inside ova was observed in many of the positive controls, but may have been too subtle to be noticed in the samples due to the greater amount of sediment and other particles obstructing observation of the ova. Counts of nematode larvae (non-motile) an order of magnitude greater than the counts of *Ascaris* ova were observed in most samples, but these data were not considered further in the study as the lab staff was not trained to make distinctions between *Ascaris* larvae and other nematodes that may be found in soil.

The pH varied within a narrow range for each vermicompost, with the minimum pH (6.66) found in the 8-month old vermicompost from San Roque, and the maximum pH (8.04) found in the 3-month old vermicompost from San Roque. The total solids content for all samples was consistently near 27%. For a numerical summary of the measurements taken in the laboratory, see **Table VIII**.

Table VIII: Laboratory Measurements									
Site, Composting Time (Number of Samples)	**Mean (*Median*) [Range] Total Ova / g TS[1]**	**Variance Total Ova / g TS**	**Mean (*Median*) [Range] Viable Ova / g TS**	**Variance Viable Ova / g TS**	**Mean (*Variance*) Ova Viability (%)**	**Mean (*Median*) [Range] pH**	**Variance pH**	**Mean (*Median*) Total Solids Content (%)**	**Variance Total Solids Content (%)**
San Roque, 3 Months (N=8)	2.30 (*1.94*) [0, 6.14]	5.65	1.02 (*0.77*) [0, 3.30]	1.49	23 (*13*)	8.04 (*7.90*) [7.77, 8.46]	0.09	27 (*27*)	0.02
San Roque, 8 Months (N=9)	9.32 (*6.35*) [0, 40.38]	155.92	7.60 (*4.76*) [0, 33.04]	109.39	65 (*12*)	6.66 (*6.68*) [6.20, 7.10]	0.07	27 (*27*)	0.06
San Roque, 13 Months (N=8)	1.74 (*2.00*) [0, 5.30]	2.99	1.43 (*0.73*) [0, 5.3]	3.68	50 (*25*)	7.14 (*7.13*) [6.79, 7.58]	0.07	30 (*28*)	0.14
Villa Mercurio, 6 Months (N=3)	1.18 (*1.63*) [0, 1.90]	1.06	1.18 (*1.63*) [0, 1.90]	1.06	100 (*0*)	7.39 (*7.28*) [7.16, 7.72]	0.09	28 (*26*)	0.08
Villa Mercurio, 18 18 Months (N=3)	10.10 (*7.65*) [0, 12.54]	39.97	1.91 (*0*) [0, 5.74]	10.97	25 (*19*)	7.92 (*7.98*) [6.97, 8.82]	0.86	25 (*26*)	0.22

1. TS - Total Solids

Modeled Distributions of Viable Ova Concentrations

Descriptive statistics of the 100,000 member probability distributions of viable ova concentrations derived from Bayesian likelihood models are shown in **Table IX**. Modeled distributions and observed distributions are shown in **Figures 5-9**, and cumulative probability distributions are shown in **Figure 10**.

Table IX: Modeled Ova Concentration Distributions

Site, Composting Time (Number of Observations)	Mean (*Median*) Viable Ova / g TS	Variance	99% Confidence Interval
San Roque, 3 Months (N=100,000)	1.12 (*0.75*)	1.73	$(1.39*10^{-3}, 6.06)$
San Roque, 8 Months (N=100,000)	6.59 (*4.62*)	49.29	$(2.09*10^{-2}, 33.08)$
San Roque, 13 Months (N=100,000)	1.41 (*0.94*)	2.88	$(5.61*10^{-4}, 7.89)$
Villa Mercurio, 6 Months (N=100,000)	1.47 (*0.87*)	3.75	$(2.51*10^{-4}, 9.02)$
Villa Mercurio, 18 Months (N=100,000)	1.96 (*0.94*)	12.17	$(5.96*10^{-9}, 15.12)$

Figure 5: Viable Ova/g TS @ San Roque 3 Month Compost

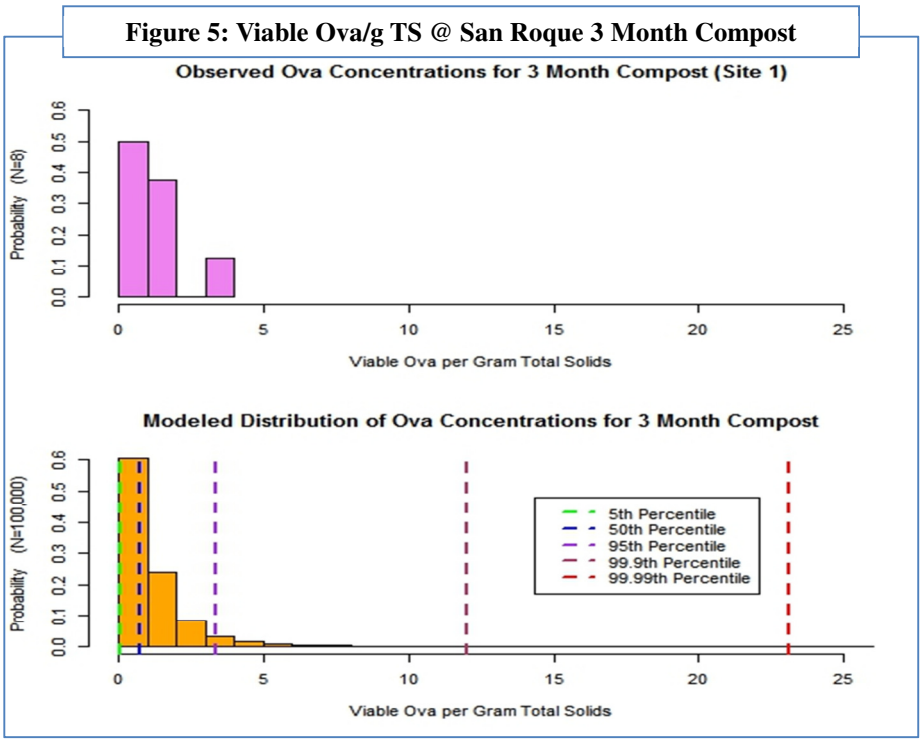


Figure 6: Viable Ova/g TS @ San Roque 8 Month Compost

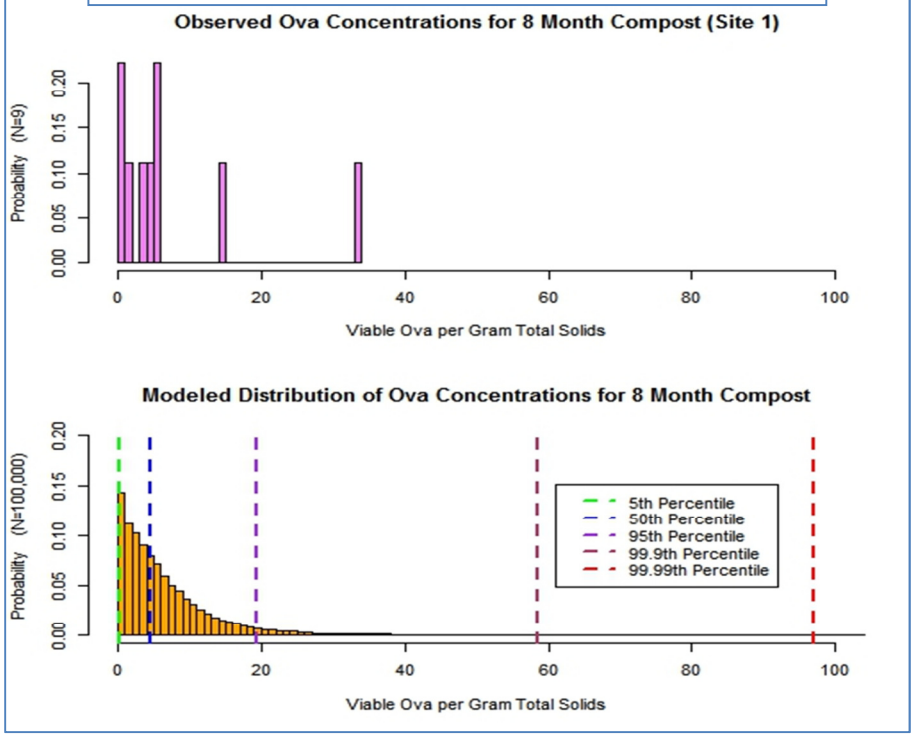


Figure 7: Viable Ova/g TS @ San Roque 13 Month Compost

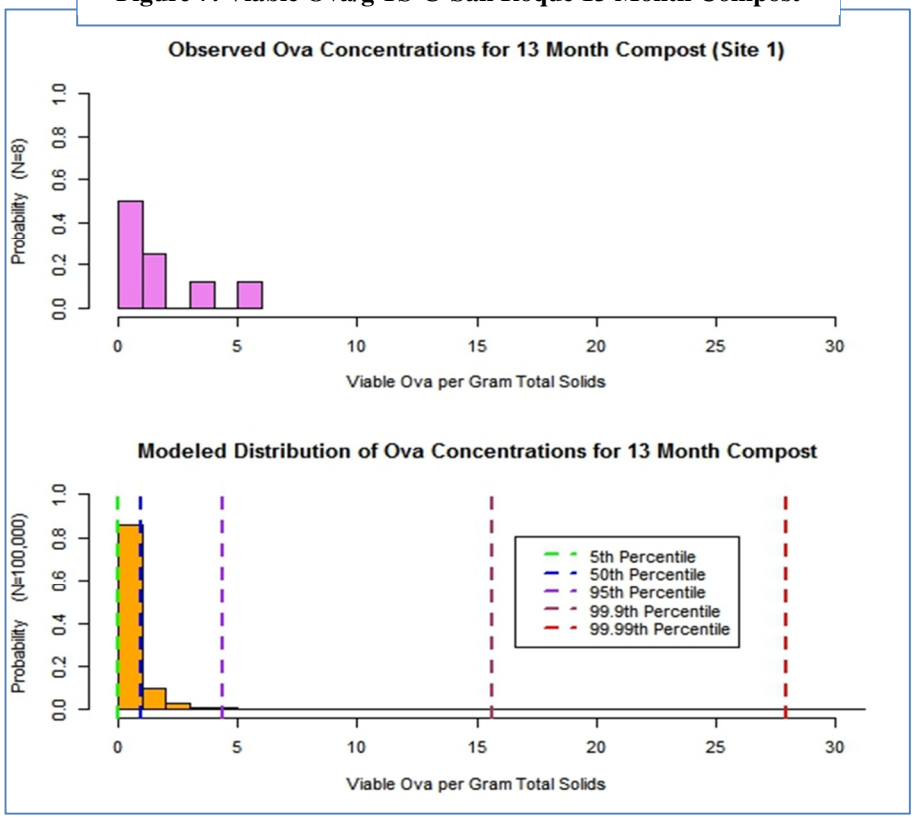


Figure 8: Viable Ova/g TS @ Villa Mercurio 6 Month Compost

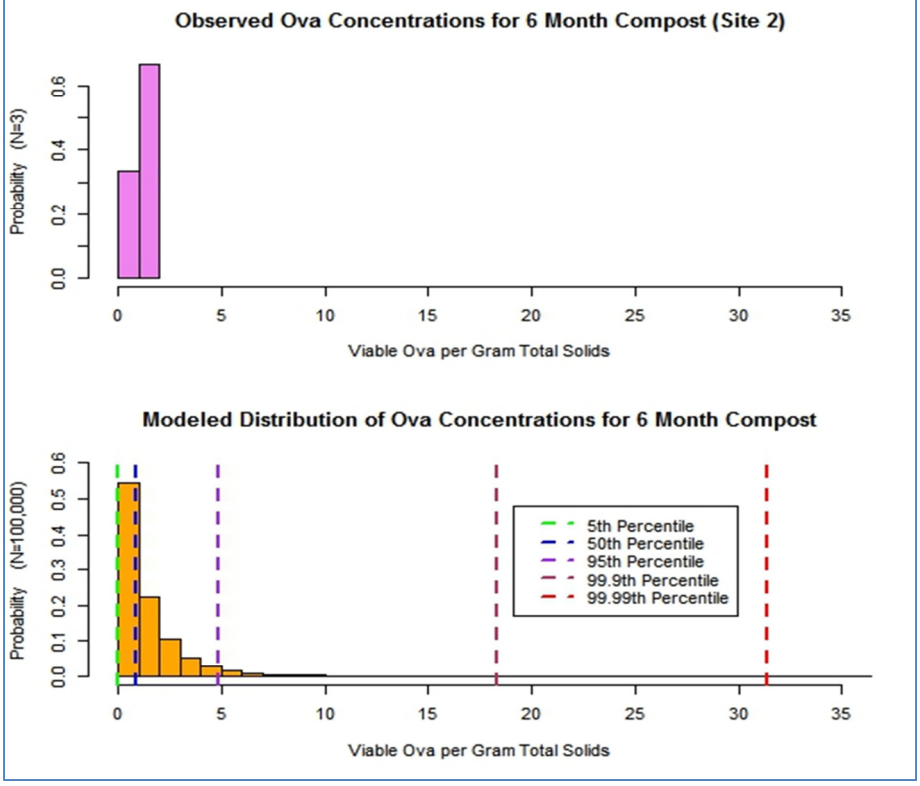


Figure 9: Viable Ova/g TS @ Villa Mercurio 18 Month Compost

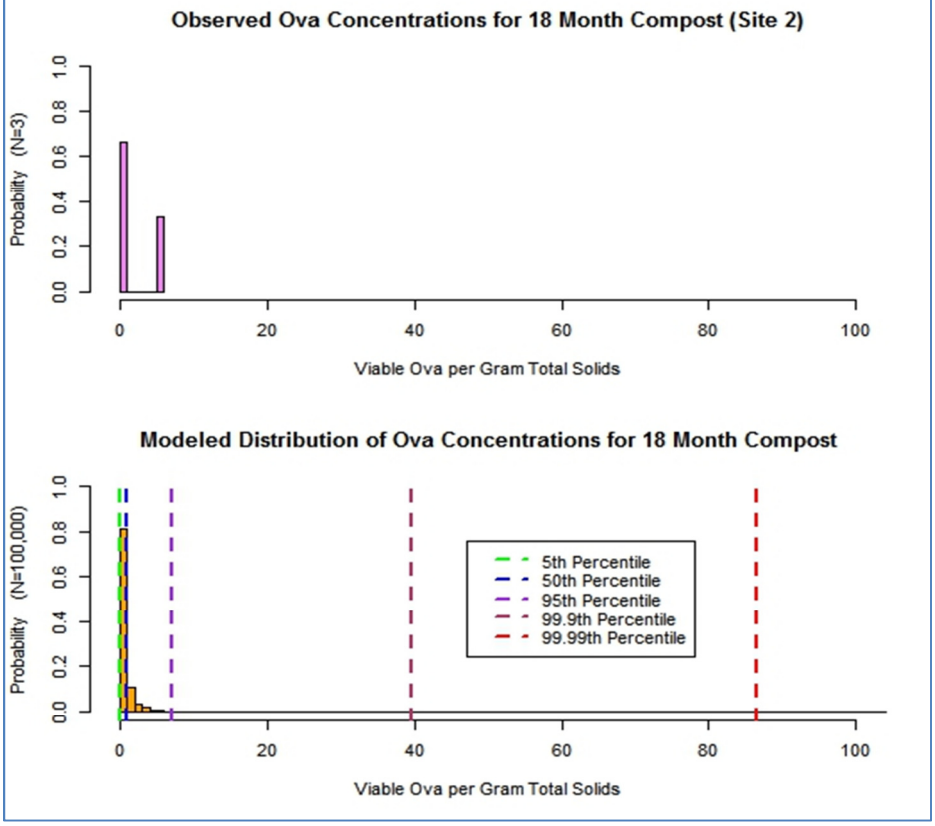
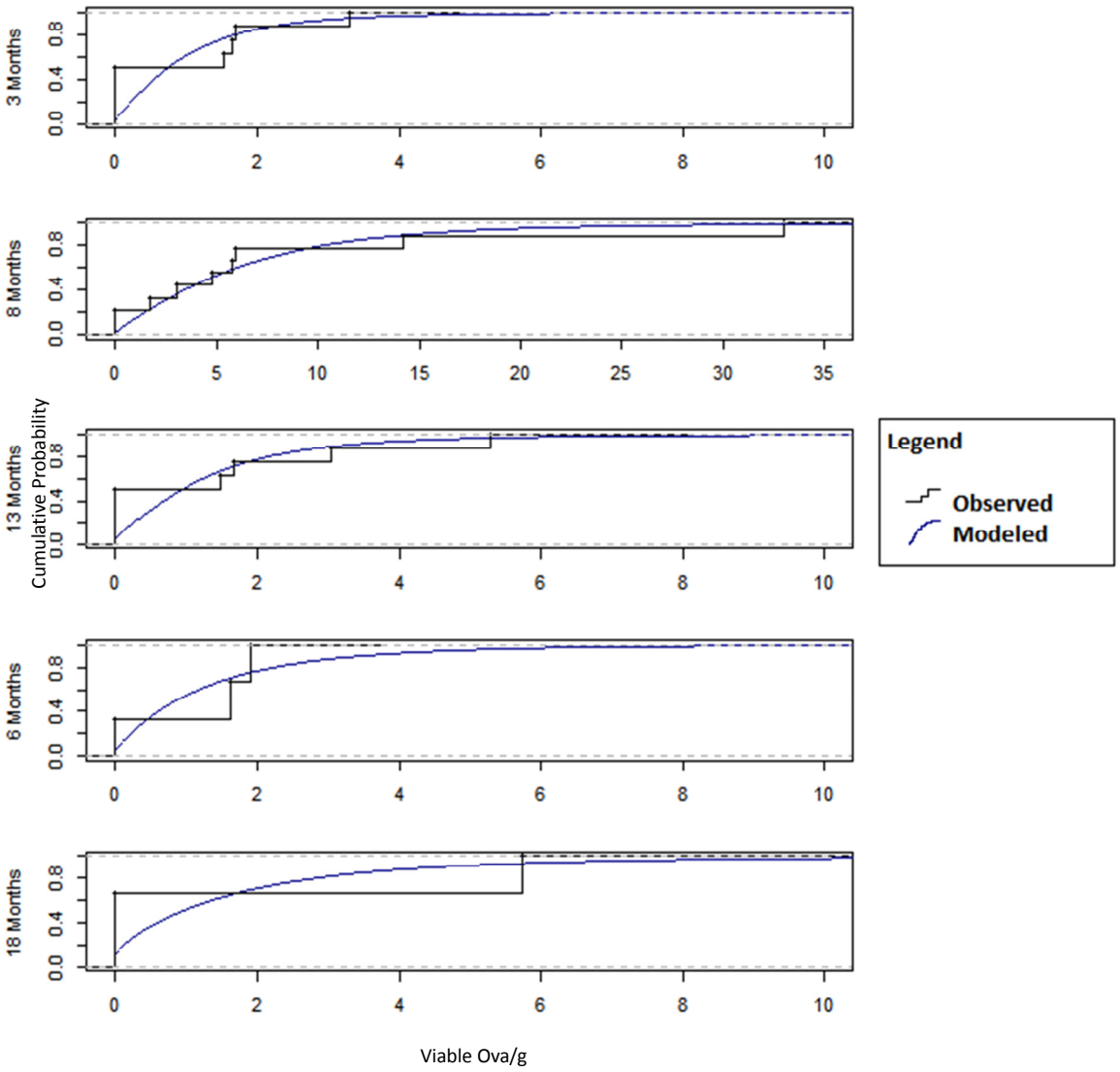


Figure 10: Cumulative Probability Plots for Observed and Modeled Concentrations of Viable Ova by Composting Time



The modeled distributions of viable ova concentrations in the composts have similar central tendencies and density functions to the observed concentrations, with the bulk of modeled and observed values falling near 0 viable ova/g TS. The modeled distributions are right-skewed, with rare values spiking as high as 157 viable ova/g TS. These tails have a significant influence on infection risks from repeated exposure.

Modeled Decay of *Ascaris* Ova in Vermicomposts

Bayesian estimates of monthly decay rates and 90% inactivation times for *Ascaris* in the vermicomposts vary widely. **Table X** summarizes the results of the Bayesian analysis of *Ascaris* ova inactivation rates in the examined composts.

Table X: Modeled Decay Rates and 90% Inactivation Times for *Ascaris* Ova in Vermicomposts

Model	Prior Assumptions	Mean (*Median*) 95% Confidence Interval for Monthly Decay Coefficient	Mean (*Median*) 95% Confidence Interval for 90% Inactivation Time (Months)
Model 1 (Initial ova concentration unknown)	Less uncertainty $\mu_3 = 0, \tau_3 = 0.1$ $\alpha_1 = 0.1, \beta_1 = 10$ $\alpha_2 = 0.1, \beta_2 = 10$ $\mu_4 = 0, \tau_4 = 0.1$	0.65 (*0.84*) (0.11, 0.999)	562.7 (*12.69*) (1.06, $2.31*10^3$)
Model 2 (Initial ova concentration set to concentration of partially developed ova at time of observation)	Less uncertainty $\mu_3 = 0, \tau_3 = 0.1$ $\alpha_1 = 0.1, \beta_1 = 10$ $\alpha_2 = 0.1, \beta_2 = 10$ $\mu_4 = 0, \tau_4 = 0.1$	0.64 (*0.79*) (0.29, 0.999)	475 (*9.8*) (1.86, $1.93*10^3$)
Model 1 (Initial ova concentration unknown)	More uncertainty $\mu_3 = 0, \tau_3 = 0.05$ $\alpha_1 = 0.05, \beta_1 = 50$ $\alpha_2 = 0.05, \beta_2 = 50$ $\mu_4 = 0, \tau_4 = 0.05$	0.72 (*0.92*) (0.14, 0.9999)	$4.56*10^4$ (*29.28*) (1.18, $3.91*10^4$)
Model 2 (Initial ova concentration set to concentration of partially developed ova at time of observation)	More uncertainty $\mu_3 = 0, \tau_3 = 0.05$ $\alpha_1 = 0.05, \beta_1 = 50$ $\alpha_2 = 0.05, \beta_2 = 50$ $\mu_4 = 0, \tau_4 = 0.05$	0.70 (*0.90*) (0.29, 0.9999)	$5.44*10^4$ (*22.63*) (1.87, $3.59*10^4$)

Assuming knowledge of the initial ova concentrations (using **Model 2**) slightly reduces the uncertainty of the estimates, but does not appreciably change mean and median estimates of the rate of inactivation. Using more or less uncertain prior assumptions on the values of parameters τ_3, τ_4, α_1, α_2, β_1, and β_2, which contribute to uncertainty in the models, also has a small effect. More uncertain assumptions regarding the values of τ_3, τ_4, α_1, α_2, β_1, and β_2 return lower estimates for the rate of inactivation of *Ascaris* in the vermicomposts (the monthly decay coefficient, or proportion surviving after each month, is raised nearer to 1). Median

estimates of the time required for 90% inactivation range from 9.8 months (**Model 2**, less uncertain prior assumptions) to 29.28 months (**Model 1**, more uncertain prior assumptions). Estimates of the 97.5th percentile time to 90% inactivation range into the thousands of months (less uncertain prior assumptions) or tens of thousands of months (more uncertain prior assumptions). This is best interpreted as showing that there is no evidence of decay at the 5% significance level in the data gathered for this study.

Risk Scenario 1: Consumption of Raw Produce Fertilized with Vermicompost

For the risk scenario involving consumption of raw spinach or carrots fertilized with vermicompost, the effects on exposure of ova concentration in the observed vermicomposts, dilution of vermicompost in soil at the soil surface, choice of function to describe inactivation of ova on crops, and reduction of ova concentrations by a tap water rinse prior to sale were analyzed. Ova concentration in the vermicomposts, dilution factor of ova at soil surface, and reduction of ova concentrations on produce by a tap water rinse prior to consumption are points where risk may be controlled by human action and regulation, whereas the function for inactivation of ova on crop surfaces is a point of uncertainty that requires scientific research to be better understood.

The estimated median daily exposures to viable *Ascaris* ova from application of any of the tested vermicomposts were between 2.5 and 15 times greater than exposures that would result from application of a compost meeting the EPA cutoff for class A biosolids (0.25 ova per gram TS) (**Table XI**). The exposures from consumption of produce fertilized with 8-month old vermicompost have median values of 1.92 ova per day for carrots, and 6.18 ova per day for spinach, and were far

higher than the exposures from the other vermicomposts (median daily exposures ranging from 0.32-0.44 ova per day for carrots and 1.01-1.43 ova per day for spinach).

The ratio to which vermicompost is diluted at the surface of the soil was directly correlated to daily exposure (**Table XII**). A 1:10 mixing ratio of vermicompost to soil resulted in roughly 1/10 the daily exposure to viable *Ascaris* ova compared to a 1:1 mixing ratio.

Assuming that *Ascaris* ova are inactivated at the same rate on crops as in soil resulted in estimated exposures that were 1.5 to 4 times higher than exposures estimated from a time independent inactivation function based on proportions of ova surviving on food items reported in the literature [102, 111] (**Table XIII**). In the time-independent inactivation model, inactivation is 2.67 times higher on carrots than it is on lettuce. It was assumed that the rate of inactivation of ova on spinach is similar to the rate reported for lettuce.

The incorporation of a tap water rinse prior to consumption of produce decreased mean daily exposures by a factor of about 10.5 and median daily exposures by a factor of about 12 (**Table XIV**).

Table XI: Effect of Viable *Ascaris* Ova Concentration in Compost on Exposure in Model for Consumption of Raw Produce			
Variable Value	**Mean** ***Median*** **(99% CI)** **Viable Ova Ingested/Day**	**Relative Effect Compared to Reference Value**	**Interpretation**
EPA Cutoff of 0.25 viable ova/g TS [12]	0.13 *0.13* (0, 0.44) 0.43 *0.41* (0, 1.38)	Reference Value	Conservative estimate of exposure with *Ascaris* ova present at the limit of detection
San Roque 3-Month Vermicompost	0.96 *0.33* (0, 11.63) 3.07 *1.06* (0, 37.09)	Mean exposure increased ~7.5 times Median exposure increased ~2.5 times 99.5th percentile exposure increased ~26.5 times	Conservative estimate of exposure with *Ascaris* ova present at levels modeled for 3-month vermicompost
San Roque 8-Month Vermicompost	4.62 *1.92* (0, 45.72) 14.86 *6.18* (0, 146.01)	Mean exposure increased ~35.5 times Median exposure increased ~15 times 99.5th percentile exposure increased ~104 times	Conservative estimate of exposure with *Ascaris* ova present at levels modeled for 8-month vermicompost

Table XI: Effect of Viable Ova Concentration in Compost on Exposure in Model for Consumption of Raw Produce (Continued)			
Variable Value	**Mean** ***Median*** **(99% CI)** **Viable Ova Ingested/Day**	**Relative Effect Compared to Reference Value**	**Interpretation**
San Roque **13-Month Vermicompost**	1.19 *0.41* (0, 14.07) 3.83 *1.32* (0, 44.48)	Mean exposure increased ~9 times Median exposure increased ~3 times 99.5th percentile exposure increased ~32 times	Conservative estimate of exposure with *Ascaris* ova present at levels modeled for 13-month vermicompost
Villa Mercurio **6-Month Vermicompost**	1.46 *0.44* (0, 18.62) 4.70 *1.43* (0, 60.11)	Mean exposure increased ~11 times Median exposure increased ~3.5 times 99.5th percentile exposure increased ~42.5 times	Conservative estimate of exposure with *Ascaris* ova present at levels modeled for 6-month vermicompost
Villa Mercurio **18-Month Vermicompost**	1.71 *0.32* (0, 28.65) 5.48 *1.01* (0, 92.31)	Mean exposure increased ~13 times Median exposure increased ~2.5 times 99.5th percentile exposure increased ~65 times	Conservative estimate of exposure with *Ascaris* ova at levels modeled for 18-month vermicompost

Table XII: Effect of Dilution of Vermicompost at Surface of Soil on Exposure in Risk Model for Consumption of Raw Produce			
Variable Value	**Mean** ***Median*** **(99% CI)** **Viable Ova Ingested/Day**	**Relative Effect Compared to Reference Value**	**Interpretation**
No Mixing, **Ratio = 1**	4.62 *1.92* (0, 45.72) 14.86 *6.18* (0, 146.01)	Reference Value	Most conservative estimate of exposure, *Ascaris* ova present at levels modeled for 8-month vermicompost. Assumes that undiluted compost is present at surface of soil.
Even Mixing, **Ratio = 0.5**	2.32 *0.96* (0, 22.65) 7.43 *3.09* (0, 72.85)	Daily exposure halved	Estimate of exposure with 50% mixing of compost with soil, *Ascaris* ova present at levels modeled for 8-month vermicompost. Represents application technique in which mixing or covering of compost with soil results in *Ascaris* ova concentrations at the surface half those found in the compost
Thorough mixing, **Ratio = 0.1**	0.46 *0.19* (0, 4.52) 1.49 *0.62* (0, 14.59)	Daily exposure reduced by a factor of 10	Estimate of exposure with 50% mixing of compost with soil, *Ascaris* ova present at levels modeled for 8-month vermicompost. Represents application technique in which mixing or covering of compost with soil results in *Ascaris* ova concentrations at the surface one tenth those found in the compost

Table XIII: Effect of Function for Inactivation of *Ascaris* Ova on Crops on Exposure in Risk Model for Consumption of Raw Produce			
Variable Value	**Mean** ***Median*** **99% CI** **Viable Ova Ingested/Day**	**Relative Effect Compared to Reference Value**	**Interpretation**
Time dependent, modeled from survival in soil Survival(x,t) = $x/(10^{t/t90})$ t90 ~ Norm (μ = 625 days, σ = 150 days)	4.62 *1.92* (0, 45.72) 14.86 *6.18* (0, 146.01)	Reference Value	Conservative estimate of exposure, *Ascaris* ova present at levels modeled for 8-month vermicompost. Assumes rate of inactivation on crops is the same as in soil.
Time independent, modeled from observed viability of ova on crops For Spinach Surviving proportion ~ Unif(0.50, 0.75) For Carrots Surviving proportion ~ Unif(0.20, 0.25)	1.20 *0.50* (0, 11.87) 10.06 *4.15* (0, 99.28)	Daily exposure for carrots decreased by roughly a factor of 4 Daily exposure for Spinach decreased by a factor of roughly 1.5	Conservative estimate of exposure, *Ascaris* ova present at levels modeled for 8-month vermicompost. Assumes constant proportions of inactivation between 0.50 and 0.75 for Spinach and 0.20 and 0.25 for carrots.

Table XIV: Effect of Tap Water Rinse on Exposure in Risk Model for Consumption of Raw Produce			
Variable Value	**Mean** ***Median*** **(99% CI)** **Viable Ova Ingested/Day**	**Relative Effect Compared to Reference Value**	**Interpretation**
No rinse	4.62 *1.92* (0, 45.72) 14.86 *6.18* (0, 146.01)	Reference Value	Conservative estimate of exposure, *Ascaris* ova present at levels modeled for 8-month vermicompost. No removal of *Ascaris* ova by rinsing.
Removal of ova with tap water rinse	0.43 *0.16* (0, 4.89) 1.41 *0.51* (0, 15.61)	Mean exposure decreased by a factor of ~10.5 Median exposure decreased by a factor of ~12 99.5^{th} percentile exposure decreased by a factor of ~9.5	Conservative estimate of exposure, *Ascaris* ova present at levels modeled for 8-month vermicompost. Tap water rinse removes a portion *Ascaris* ova prior to sale of produce.

The dose-response model and the number of days contaminated produce may be consumed each year convert exposure to daily and annual infection risk in the model for consumption of raw produce fertilized with vermicompost. The most realistic values for these parameters are uncertain, so the relative effects of the exponential and beta-Poisson dose-response functions, as well as having 56 or 365 days of exposure per year were quantified with respect to risk.

The choice of the dose-response model had a strong influence on estimated daily and annual risks of infection. The Beta-Poisson dose response model estimated daily risks that were an order of magnitude lower than those estimated by the exponential dose-response model given exposures corresponding to application of the 8-month old vermicompost mixed to 10% at the surface of the soil and a tap water rinse of produce prior to consumption. As a result of the decreased daily risks, the annual risks of infection were substantially lower with the beta-Poisson dose-response model than with the exponential model (**Table XV**).

The impact of the number of days of exposure to produce grown with vermicompost on estimated infection risks was mediated by the choice of the dose-response model (**Table XVI**). The infection risks resulting from the exponential dose-response model approach 100% at fairly low exposures, and the model is therefore less sensitive to increases in exposure or exposure frequency outside of a narrow range of values. When the exponential dose-response model was used, annual risks of infection with 56 days of exposure were about 25% lower than with 365 days of exposure. When the beta-Poisson dose-response model was used, annual risks of infection were about 44.5% lower with 56 days of exposure than with 365 days of exposure.

Table XV: Effect of Dose-Response Model on Estimated *Ascaris* Infection Risk from Consumption of Produce Fertilized with Vermicompost			
Variable Value	**Mean** ***Median*** **(99% CI)** **% Risk of Infection**	**Relative Effect Compared to Reference Value**	**Interpretation**
Exponential Dose-Response Model	Daily Risk: 2.40 *0.87* (0, 25.20) Annual Risk (365 Days): 99.99 *99.99* (99.924, 99.999)	Reference Value	Less conservative model (10% mix ratio, rinse, carrots), *Ascaris* ova present at 8-month vermicompost levels. Exposure is Poisson distributed, single ova ingested causes infection 100% of time
Beta-Poisson Dose-Response Model	Daily Risk: 0.23 *0.08* (0, 2.41) Annual Risk (365 Days): 56.56 *56.53* (48.19, 65.29)	Daily risks reduced by a factor of ~10.5 Annual risk reduced by 34.7-51.8% (Effect size will vary as exposure changes, as dose-response functions have different slopes)	Less conservative model (10% soil mixing, detergent wash, carrots), *Ascaris* ova present at levels modeled for 8-month vermicompost. Estimated from Epidemiologic data among children aged 5-15 in Mexico. May not be applicable to other settings and age groups.

Table XVI: Effect of Exposure Frequency on Modeled Infection Risk from Consumption of Produce Fertilized with Vermicompost (Stratified by Dose-Response Model)			
Variable Value	**Mean** ***Median*** **(99% CI)** **% Risk of Infection**	**Relative Effect Compared to Reference Value**	**Interpretation**
365 Days of Possible Exposure	Annual Risk (Exp Dose-Resp) 99.99 *99.99* (99.924, 99.999) Annual Risk (B-Pois Dose-Resp) 56.56 *56.53* (48.19, 65.29)	Reference Value	Less conservative model (10% mix ratio, rinse, carrots), *Ascaris* ova present at 8-month vermicompost levels. Assumes consumers may ingest raw produce grown with vermicompost every day of the year.
56 Days of Possible Exposure	Annual Risk (Exp Dose-Resp) 74.21 *74.63* (50.37, 92.72) Annual Risk (B-Pois Dose-Resp) 12.03 *11.78* (6.34, 20.36)	Decrease central tendency annual risks 25%, increase variability in risk. Decrease central tendency annual risks 44.5% with beta-Poisson dose-response curve	Less conservative model (10% mix ratio, rinse, carrots), *Ascaris* ova present at 8-month vermicompost levels. Assumes that produce grown with vermicompost will be available eight weeks per year.

In order to examine the worst-case and best-case scenario estimates of annual risk of *Ascaris* infection from consumption of raw produce grown with vermicompost, the modeled concentrations of viable *Ascaris* ova in the 8-month old compost were used along with 365 days of exposure per year as a basis for the worst-case estimates of risk, and the modeled concentrations of viable *Ascaris* ova in the 3-month old compost were used along with 56 days of exposure per year as the basis for best-case estimates of risk. For these scenarios, it was assumed that the function describing inactivation of the ova on crops should be the time-independent function derived from observations of *Ascaris* ova viability on carrots and lettuce in the literature. The ratio of dilution of vermicompost in soil at the surface and the inclusion of a tap water rinse represent control points where policy may influence the risk of infection and the effect of these parameters on risk was included in the analysis. Both the exponential and beta-Poisson dose-response functions were applied to each model to give the upper and lower risk estimates.

The best-case and worst-case models estimated very high annual risks of *Ascaris* infection. Under the worst-case model, annual risks of infection remained near 100% unless vermicompost was mixed to 10% at the surface of soil, a tap water rinse was implemented, and the beta-Poisson dose-response model was used. Even under the best-case assumptions, the lowest median annual risk was 2.53% (for carrots grown with a 10% dilution of vermicompost in soil and a tap water rinse before consumption). Daily risks under the beta-Poisson dose-response model tended to be about 90% lower than when the exponential dose-response model was used (**Tables XVII-XX**).

Table XVII: Worst-Case *Ascaris* Infection Risk Distributions for Consumption of Raw Carrots (1000 Simulations)					
Model Description	**Control Points**	**Median (99% Conf. Interval) Daily Risk (%), Exponential Dose-Response**	**Median (99% Conf. Interval) Annual Risk (%), Exponential Dose-Response**	**Median (99% Conf. Interval) Daily Risk (%), Beta-Poisson Dose-Response**	**Median (99% Conf. Interval) Annual Risk (%), Beta-Poisson Dose-Response**
Consumption of raw carrots fertilized with compost containing ova concentrations modeled for eight-month old vermicompost (highest) with 365 days of possible exposure	Compost/Soil Ratio: 100% No Rinse	65.90 (0, 100)	100 (100, 100)	6.86 (0, 28.97)	100 (100, 100)
	Compost/Soil Ratio: 50% No Rinse	41.60 (0, 100)	100 (100, 100)	4.07 (0, 23.95)	100 (100, 100)
	Compost/Soil Ratio: 10% No Rinse	10.20 (0, 94.11)	100 (100, 100)	0.97 (0, 12.43)	99.90 (99.70, 99.97)
	Compost/Soil Ratio: 10% Tap Water Rinse	0.09 (0, 26.08)	99.99 (99.92, 100)	0.08 (0, 2.50)	56.38 (48.58, 65.40)

Table XVIII: Worst-Case *Ascaris* Infection Risk Distributions for Consumption of Raw Spinach (1000 Simulations)					
Model Description	**Control Points**	**Median (99% Conf. Interval) Daily Risk (%), Exponential Dose-Response**	**Median (99% Conf. Interval) Annual Risk (%), Exponential Dose-Response**	**Median (99% Conf. Interval) Daily Risk (%), Beta-Poisson Dose-Response**	**Median (99% Conf. Interval) Annual Risk (%), Beta-Poisson Dose-Response**
Consumption of raw spinach fertilized with compost containing ova concentrations modeled for eight-month old vermicompost (highest) with 365 days of possible exposure	Compost/Soil Ratio: 100% No Rinse	96.14 (0, 100)	100 (100, 100)	13.35 (0, 36.50)	100 (100, 100)
	Compost/Soil Ratio: 50% No Rinse	80.36 (0, 100)	100 (100, 100)	9.03 (0, 31.84)	100 (100, 100)
	Compost/Soil Ratio: 10% No Rinse	27.78 (0, 99.98)	100 (100, 100)	2.67 (0, 20.22)	100 (100, 100)
	Compost/Soil Ratio: 10% Tap Water Rinse	2.65 (0, 59.44)	100 (100, 100)	0.25 (0, 6.05)	90.23 (84.83, 94.20)

Table XIX: Best-Case *Ascaris* Infection Risk Distributions for Consumption of Raw Carrots (1000 Simulations)					
Model Description	**Control Points**	**Median (99% Conf. Interval) Daily Risk (%), Exponential Dose-Response**	**Median (99% Conf. Interval) Annual Risk (%), Exponential Dose-Response**	**Median (99% Conf. Interval) Daily Risk (%), Beta-Poisson Dose-Response**	**Median (99% Conf. Interval) Annual Risk (%), Beta-Poisson Dose-Response**
Consumption of raw carrots fertilized with compost containing ova concentrations modeled for three-month old vermicompost (lowest) with 56 days of possible exposure	Compost/Soil Ratio: 100% No Rinse	65.90 (0, 100)	100 (100, 100)	6.86 (0, 28.97)	82.47 (66.86, 92.72)
	Compost/Soil Ratio: 50% No Rinse	41.60 (0, 100)	100 (99.95, 100)	4.07 (0, 23.95)	63.70 (45.53, 80.46)
	Compost/Soil Ratio: 10% No Rinse	10.20 (0, 94.11)	94.87 (77.80, 99.79)	0.97 (0, 12.43)	22.44 (11.65, 40.24)
	Compost/Soil Ratio: 10% Tap Water Rinse	0.88 (0, 26.08)	23.95 (13.85, 49.32)	0.08 (0, 2.5)	2.53 (1.17, 6.66)

Table XX: Best-Case *Ascaris* Infection Risk Distributions for Consumption of Raw Spinach (1000 Simulations)					
Model Description	**Control Points**	**Median (99% Conf. Interval) Daily Risk (%), Exponential Dose-Response**	**Median (99% Conf. Interval) Annual Risk (%), Exponential Dose-Response**	**Median (99% Conf. Interval) Daily Risk (%), Beta-Poisson Dose-Response**	**Median (99% Conf. Interval) Annual Risk (%), Beta-Poisson Dose-Response**
Consumption of raw spinach fertilized with compost containing ova concentrations modeled for three-month old vermicompost (lowest) with 56 days of possible exposure	Compost/Soil Ratio: 100% No Rinse	96.14 (0, 100)	100 (100, 100)	13.35 (0, 36.5)	97.20 (90.40, 99.28)
	Compost/Soil Ratio: 50% No Rinse	80.36 (0, 100)	100 (100, 100)	9.03 (0, 31.84)	90.37 (76.89, 96.46)
	Compost/Soil Ratio: 10% No Rinse	27.78 (0, 99.98)	99.99 (99.24, 100)	2.67 (0, 20.22)	49.70 (34.38, 66.03)
	Compost/Soil Ratio: 10% Tap Water Rinse	2.65 (0, 59.44)	56.38 (33.73, 81.62)	0.25 (0, 6.05)	7.54 (4.00, 16.43)

The burden of disease for the best and worst-case models for *Ascaris* infection risk from consumption of raw produce fertilized with vermicompost was calculated according to the method adapted from Bundy, Chan *et al* (see methods and literature review) [96]. When compared to a WHO proposed cutoff of 10^{-4} DALYs per person per year for an acceptable burden of disease due to reuse of excreta [14], every scenario produced an unacceptable burden of disease with the exception of the best-case scenario for consumption of raw carrots fertilized with vermicompost, which assumes use of vermicompost with *Ascaris* ova concentrations equivalent to those modeled for the 3-month old vermicompost, availability of produce grown with the vermicompost for 56 days per year, dilution of vermicompost to 10% in surface soil, a tap water rinse prior to sale of the produce, and applies the Beta-Poisson dose-response model. This best-case scenario produced a median burden of disease of $1.64*10^{-5}$ DALYs per person per year, significantly less than the 10^{-4} DALYs per person per year cutoff at the 95% confidence level (**Tables XXI-XXIV**).

Table XXI: Burden of Disease for Worst-Case *Ascaris* Infection Risk From Consumption of Raw Carrots (1000 Simulations)			
Model Description	**Control Points**	**Median (95% Conf. Interval) Burden of Disease Exponential Dose-Response (DALYs per person per year)**	**Median (95% Conf. Interval) Burden of Disease Beta-Poisson Dose-Response (DALYs per person per year)**
Consumption of raw carrots fertilized with compost containing ova concentrations modeled for eight-month old vermicompost (highest) with 365 days of possible exposure	Compost/Soil Ratio: 100% No Rinse	$1.59*10^{-1}$ ($1.59*10^{-1}$, $1.59*10^{-1}$)	$1.59*10^{-1}$ ($1.59*10^{-1}$, $1.59*10^{-1}$)
	Compost/Soil Ratio: 50% No Rinse	$1.59*10^{-1}$ ($1.59*10^{-1}$, $1.59*10^{-1}$)	$1.59*10^{-1}$ ($1.59*10^{-1}$, $1.59*10^{-1}$)
	Compost/Soil Ratio: 10% No Rinse	$1.59*10^{-1}$ ($1.59*10^{-1}$, $1.59*10^{-1}$)	$1.59*10^{-1}$ ($1.59*10^{-1}$, $1.59*10^{-1}$)
	Compost/Soil Ratio: 10% Tap Water Rinse	$1.59*10^{-1}$ ($1.59*10^{-1}$, $1.59*10^{-1}$)	$8.27*10^{-2}$ ($7.80*10^{-2}$, $8.77*10^{-2}$)

Table XXII: Burden of Disease for Worst-Case *Ascaris* Infection Risk From Consumption of Raw Spinach (1000 Simulations)			
Model Description	**Control Points**	**Median (95% Conf. Interval) Burden of Disease Exponential Dose-Response (DALYs per person per year)**	**Median (95% Conf. Interval) Burden of Disease Beta-Poisson Dose-Response (DALYs per person per year)**
Consumption of raw spinach fertilized with compost containing ova concentrations modeled for eight-month old vermicompost (highest) with 365 days of possible exposure	Compost/Soil Ratio: 100% No Rinse	$1.59*10^{-1}$ ($1.59*10^{-1}$, $1.59*10^{-1}$)	$1.59*10^{-1}$ ($1.59*10^{-1}$, $1.59*10^{-1}$)
	Compost/Soil Ratio: 50% No Rinse	$1.59*10^{-1}$ ($1.59*10^{-1}$, $1.59*10^{-1}$)	$1.59*10^{-1}$ ($1.59*10^{-1}$, $1.59*10^{-1}$)
	Compost/Soil Ratio: 10% No Rinse	$1.59*10^{-1}$ ($1.59*10^{-1}$, $1.59*10^{-1}$)	$1.59*10^{-1}$ ($1.59*10^{-1}$, $1.59*10^{-1}$)
	Compost/Soil Ratio: 10% Tap Water Rinse	$1.59*10^{-1}$ ($1.59*10^{-1}$, $1.59*10^{-1}$)	$1.41*10^{-1}$ ($1.39*10^{-1}$, $1.44*10^{-1}$)

Table XXIII: Burden of Disease for Best-Case *Ascaris* Infection Risk From Consumption of Raw Carrots (1000 Simulations)			
Model Description	**Control Points**	**Median (95% Conf. Interval) Burden of Disease Exponential Dose-Response (DALYs per person per year)**	**Median (95% Conf. Interval) Burden of Disease Beta-Poisson Dose-Response (DALYs per person per year)**
Consumption of raw carrots fertilized with compost containing ova concentrations modeled for three-month old vermicompost (lowest) with 56 days of possible exposure	Compost/Soil Ratio: 100% No Rinse	$1.59*10^{-1}$ ($1.59*10^{-1}$, $1.59*10^{-1}$)	$1.27*10^{-1}$ ($1.19*10^{-1}$, $1.35*10^{-1}$)
	Compost/Soil Ratio: 50% No Rinse	$1.59*10^{-1}$ ($1.59*10^{-1}$, $1.59*10^{-1}$)	$9.60*10^{-2}$ ($8.61*10^{-2}$, $1.06*10^{-1}$)
	Compost/Soil Ratio: 10% No Rinse	$1.48*10^{-1}$ ($1.40*10^{-1}$, $1.53*10^{-1}$)	$2.56*10^{-2}$ ($1.91*10^{-2}$, $3.25*10^{-2}$)
	Compost/Soil Ratio: 10% Tap Water Rinse	$2.89*10^{-2}$ ($2.17*10^{-2}$, $3.91*10^{-2}$)	$1.64*10^{-5}$ ($5.23*10^{-6}$, $9.62*10^{-5}$)

Table XXIV: Burden of Disease for Best-Case *Ascaris* Infection Risk From Consumption of Raw Spinach (1000 Simulations)			
Model Description	**Control Points**	**Median (95% Conf. Interval) Burden of Disease Exponential Dose-Response (DALYs per person per year)**	**Median (95% Conf. Interval) Burden of Disease Beta-Poisson Dose-Response (DALYs per person per year)**
Consumption of raw spinach fertilized with compost containing ova concentrations modeled for three-month old vermicompost (lowest) with 56 days of possible exposure	Compost/Soil Ratio: 100% No Rinse	$1.59*10^{-1}$ ($1.59*10^{-1}$, $1.59*10^{-1}$)	$1.54*10^{-1}$ ($1.50*10^{-1}$, $1.55*10^{-1}$)
	Compost/Soil Ratio: 50% No Rinse	$1.59*10^{-1}$ ($1.59*10^{-1}$, $1.59*10^{-1}$)	$1.41*10^{-1}$ ($1.36*10^{-1}$, $1.46*10^{-1}$)
	Compost/Soil Ratio: 10% No Rinse	$1.59*10^{-1}$ ($1.59*10^{-1}$, $1.59*10^{-1}$)	$7.11*10^{-2}$ ($6.16*10^{-2}$, $8.12*10^{-2}$)
	Compost/Soil Ratio: 10% Tap Water Rinse	$8.25*10^{-2}$ ($6.80*10^{-2}$, $9.68*10^{-2}$)	$2.41*10^{-3}$ ($1.15*10^{-3}$, $5.36*10^{-3}$)

For the purpose of defining a measure for adequately sanitized Ecosan composts, the maximum concentration of ova in vermicompost that would result in an acceptable burden of disease (below 10^{-4} DALYs per person per year) was modeled. As the long-term viability of the ecological sanitation strategy may be best served through sale of composts to farmers year-round, it was deemed most appropriate to model the maximum allowable concentration of ova in vermicompost with 365 days of exposure, along with best-case control points of 10% dilution of compost at the surface of soil and a tap water rinse prior to consumption.

The maximum allowable ova concentration in vermicompost for growing carrots was calculated to be about 0.02 viable ova per gram when using the exponential dose-response model and about 0.21 ova per gram when using the beta-Poisson dose-response model. For growing spinach, the maximum allowable ova concentration in vermicompost was calculated to be about 0.003 viable ova per gram when using the exponential dose-response model and about 0.026 viable ova per gram when using the beta-Poisson dose-response model.

The EPA cutoff for class A biosolids is 0.25 ova per gram TS and is taken from the lower limit of detection of the method used here for recovering and quantifying *Ascaris* in biosolids [12]. Considering the moisture content of the vermicomposts examined in this study, this corresponds to about 0.075 ova per gram vermicompost. Among the four models examined here, acceptable burdens of disease at ova concentrations above the limit of detection only occur when using the model for carrot consumption with the beta-Poisson dose-response formula (see **Figures 11-12**).

Figure 11: Burden of Disease by Ova Concentration for Carrots

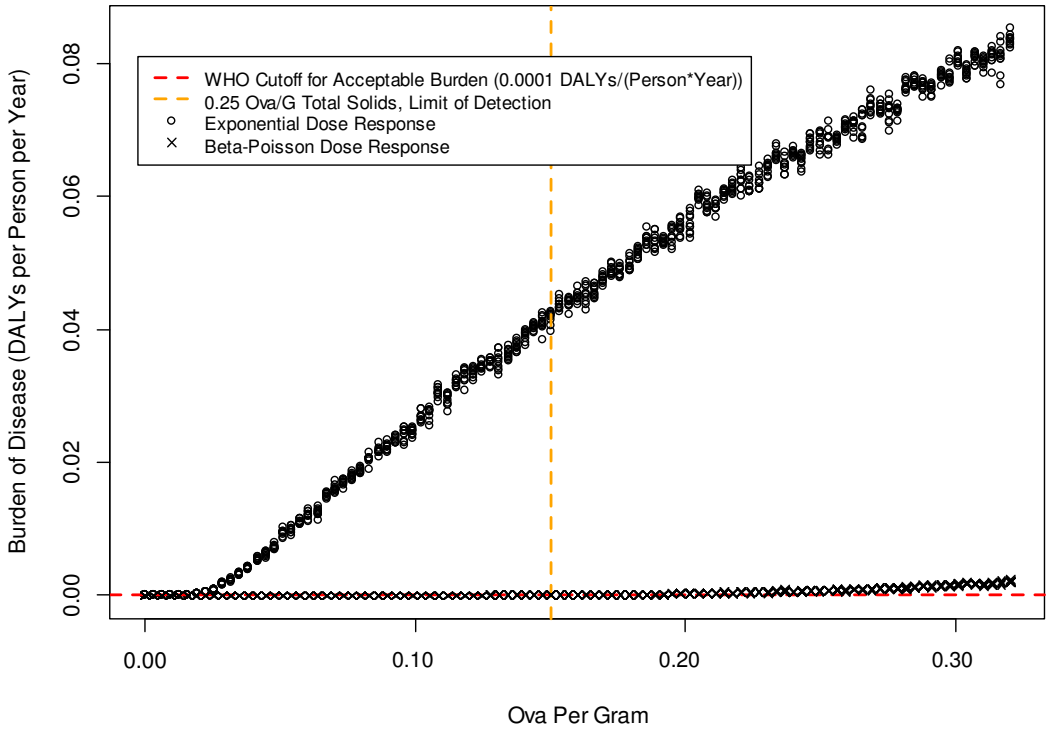
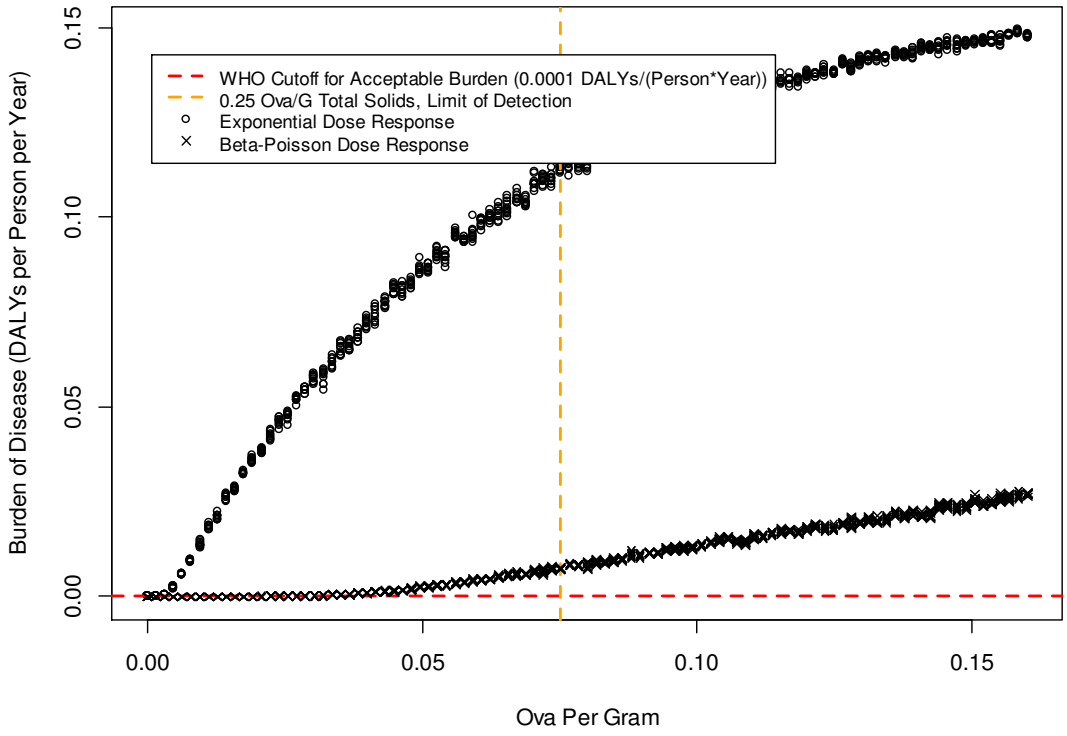


Figure 12: Burden of Disease by Ova Concentration for Spinach



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Risk Scenario 2: Soil Ingestion by Agricultural Workers at Sites Fertilized with Vermicompost

For the risk scenario involving accidental ingestion of soil by agricultural workers laboring on fields fertilized with vermicompost, the effects on exposure of the concentration of viable *Ascaris* ova in the vermicompost and the dilution of vermicompost in soil at the soil surface were analyzed.

The estimated median daily exposures to viable *Ascaris* ova from application of the tested vermicomposts ranged from 0.009 ova per day (3-month old, 18-month old vermicomposts) to 0.048 ova per day (8-month old vermicompost). The estimated median exposures from the tested composts were between 3 and 17 times greater than the estimated median exposure from application of a compost meeting the EPA cutoff for class A biosolids (0.25 ova per gram TS) (**Table XXV**). The estimated median exposures for this scenario were reduced by more than an order of magnitude from those estimated for the scenario of consumption of raw produce grown with vermicompost.

Diluting the vermicompost in soil at the soil surface to 50% or 10% reduces mean and median estimated daily exposures to viable *Ascaris* ova by roughly 50% or 90% (**Table XXVI**).

Table XXV: Effect of Viable Ova Concentration in Compost on Exposure in Risk Model for Accidental Ingestion of Soil by Agricultural Workers			
Variable Value	**Mean** ***Median*** **(99% CI)** **Viable Ova Ingested/Day**	**Relative Effect Compared to Reference Value**	**Interpretation**
EPA Cutoff of 0.25 viable ova/g TS	0.0028 *0.0028* ($5.12*10^{-4}$, $5.49*10^{-3}$)	Reference Value	Conservative estimate of exposure with *Ascaris* ova present at the limit of detection
San Roque 3-Month Vermicompost	0.018 *0.009* ($1.67*10^{-8}$, 0.18)	Mean exposure increased ~6.5 times Median exposure increased ~3 times 99.5^{th} percentile exposure increased ~33 times	Conservative estimate of exposure with *Ascaris* ova present at levels modeled for 3-month vermicompost
San Roque 8-Month Vermicompost	0.084 *0.048* ($7.47*10^{-6}$, 0.56)	Mean exposure increased ~30 times Median exposure increased ~17 times 99.5^{th} percentile exposure increased ~102 times	Conservative estimate of exposure with *Ascaris* ova present at levels modeled for 8-month vermicompost
San Roque 13-Month Vermicompost	0.024 *0.011* ($2.22*10^{-8}$, 0.23)	Mean exposure increased ~8.5 times Median exposure increased ~4 times 99.5^{th} percentile exposure increased ~42 times	Conservative estimate of exposure with *Ascaris* ova present at levels modeled for 13-month vermicompost
Villa Mercurio 6-Month Vermicompost	0.03 *0.012* ($2.18*10^{-9}$, 0.30)	Mean exposure increased ~10.5 times Median exposure increased ~4.5 times 99.5^{th} percentile exposure increased ~54.5 times	Conservative estimate of exposure with *Ascaris* ova present at levels modeled for 6-month vermicompost
Villa Mercurio 18-Month Vermicompost	0.031 *0.009* (0, 0.38)	Mean exposure increased ~11 times Median exposure increased ~3 times 99.5^{th} percentile exposure increased ~69 times	Conservative estimate of exposure with *Ascaris* ova at levels modeled for 18-month vermicompost

Table XXVI: Effect of Dilution of Vermicompost at Surface of Soil on Exposure in Risk Model for Soil Ingestion by Agricultural Workers			
Variable Value	**Mean** ***Median*** **(99% CI)** **Viable Ova Ingested/Day**	**Relative Effect Compared to Reference Value**	**Interpretation**
No Mixing, **Ratio = 1**	0.084 *0.048* ($7.47*10^{-6}$, 0.56)	Reference Value	Most conservative estimate of exposure, *Ascaris* ova present at levels modeled for 8 month vermicompost. Assumes that undiluted compost is present at surface of soil.
Even Mixing, **Ratio = 0.5**	0.045 *0.024* ($4.35*10^{-6}$, 0.33)	Daily exposure halved	Estimate of exposure with 50% mixing of compost with soil, *Ascaris* ova present at levels modeled for 8 month vermicompost. Represents application technique in which mixing or covering of compost with soil results in *Ascaris* ova concentrations at the surface half those found in the compost
Thorough mixing, **Ratio = 0.1**	0.0095 *0.0049* ($6.56*10^{-7}$, 0.075)	Daily exposure reduced by a factor of 10	Estimate of exposure with 50% mixing of compost with soil, *Ascaris* ova present at levels modeled for 8 month vermicompost. Represents application technique in which mixing or covering of compost with soil results in *Ascaris* ova concentrations at the surface one tenth those found in the compost

The choice of dose-response model strongly influences estimates of daily and annual risks of *Ascaris* infection for agricultural workers laboring on fields fertilized with vermicompost. Daily infection risks estimated by the beta-Poisson dose-response model were roughly 5 times lower than those estimated by the exponential dose-response model (**Table XXVII**). This effect was decreased from the risk models for consumption of produce grown with vermicompost because the estimated exposures for agricultural workers are lower and fall in a range where there is less difference in the estimates provided by the exponential and beta-Poisson dose response models.

Table XXVII: Effect of Dose-Response Model on Estimated *Ascaris* Infection Risk for Agricultural Workers Laboring on Fields Fertilized with Vermicompost

Variable Value	Mean *Median* (99% CI) % Risk of Infection	Relative Effect Compared to Reference Value	Interpretation
Exponential Dose-Response Model	Daily Risk: 4.47 *2.39* (0, 34.46) Annual Risk (126 Days): 99.71 *99.77* (98.696, 99.979)	Reference Value	Conservative estimate of exposure (no soil mixing), *Ascaris* ova present at levels modeled for 8-month vermicompost. Exposure is Poisson distributed, single ova ingested causes infection 100% of time
Beta-Poisson Dose-Response Model	Daily Risk: 0.82 *0.47* (0, 5.75) Annual Risk (126 Days): 64.43 *64.44* (54.02, 73.52)	Daily risks reduced by a factor of ~5 Annual risk reduced by 25.2-46% (Effect size will vary as exposure changes, as dose-response functions have different slopes)	Conservative estimate of exposure (no soil mixing), *Ascaris* ova present at levels modeled for 8-month vermicompost. Estimated from Epidemiologic data among children aged 5-15 in Mexico. May not be applicable to other settings and age groups.

In order to examine the worst-case and best-case estimates of annual risk of *Ascaris* infection to agricultural workers laboring on sites fertilized with vermicompost, the highest and lowest ova concentrations modeled for the observed composts were used in the risk model (concentrations modeled from 8-month old vermicompost and 3-month old vermicompost, respectively). The ratio of dilution of vermicompost in soil at the surface was set as a control point, and its effect was analyzed for each model. The exponential and beta-Poisson dose-response functions were applied to each model to give upper and lower estimates of risk.

The median annual risks of *Ascaris* infection estimated by the worst-case model ranged from 10.78% to 99.77%, with corresponding median daily risks of 0.05% to 4.82% (**Table XXVIII**). Median annual risks estimated by the best-case model ranged from 2.30% to 70.79%, with corresponding median daily risks of 0.008% to 0.88% (**Table XXIX**).

Table XXVIII: Worst-Case *Ascaris* Infection Risk Distributions for Worker Soil Ingestion (1000 Simulations)					
Model Description	**Control Points**	**Median (99% Conf. Interval) Daily Risk (%), Exponential Dose-Response**	**Median (99% Conf. Interval) Annual Risk (%), Exponential Dose-Response**	**Median (99% Conf. Interval) Daily Risk (%), Beta-Poisson Dose-Response**	**Median (99% Conf. Interval) Annual Risk (%), Beta-Poisson Dose-Response**
Ingestion of soil fertilized with compost containing ova concentrations modeled for eight-month old vermicompost, 126 days of exposure	Compost/Soil Ratio 100%	4.82 (0, 56.6)	99.77 (98.7, 99.98)	0.47 (0, 5.63)	64.44 (54.02, 73.52)
	Compost/Soil Ratio 50%	2.45 (0, 32.49)	99.77 (98.7, 99.98)	0.23 (0, 3.25)	42.11 (33.74, 53.26)
	Compost/Soil Ratio 10%	0.50 (0, 8.66)	70.83 (58.94, 81.31)	0.05 (0, 0.78)	10.78 (8.00, 14.55)

Table XXIX: Best-Case *Ascaris* Infection Risk Distributions for Worker Soil Ingestion (1000 Simulations)					
Model Description	**Control Points**	**Median (99% Conf. Interval) Daily Risk (%), Exponential Dose-Response**	**Median (99% Conf. Interval) Annual Risk (%), Exponential Dose-Response**	**Median (99% Conf. Interval) Daily Risk (%), Beta-Poisson Dose-Response**	**Median (99% Conf. Interval) Annual Risk (%), Beta-Poisson Dose-Response**
Ingestion of soil fertilized with compost containing ova concentrations modeled for three-month old vermicompost, 126 days of possible exposure	Compost/Soil Ratio 100%	0.88 (0, 19.77)	70.79 (57.61, 86.17)	0.08 (0, 1.79)	20.38 (14.71, 27.34)
	Compost/Soil Ratio 50%	0.44 (0, 10.45)	70.31 (57.09, 84.72)	0.04 (0, 1.01)	10.85 (7.51, 16.28)
	Compost/Soil Ratio 10%	0.09 (0, 1.90)	21.93 (15.44, 31.49)	0.008 (0, 0.20)	2.30 (1.56, 3.53)

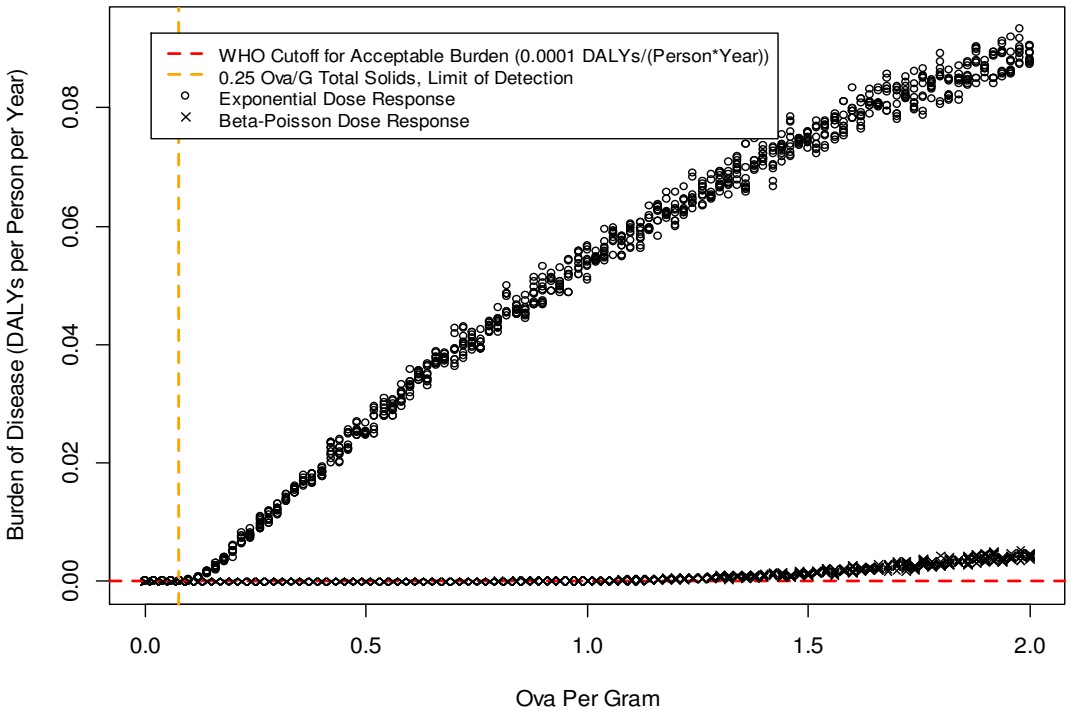
The burden of disease for the best and worst-case scenarios was calculated according to the method adapted from Bundy, Chan *et al* [96]. When compared to a WHO proposed cutoff of 10^{-4} DALYs per person per year for an acceptable burden of disease due to reuse of excreta, the only scenario producing an acceptable burden was the best-case model using viable *Ascaris* ova concentrations modeled from the 3-month old compost, 10% dilution of vermicompost at the surface of soil, and the beta-Poisson dose-response model. This best-case scenario produced a median burden of disease of $6.05*10^{-7}$ DALYs per person per year, which was significantly less than the 10^{-4} DALYs per person per year cutoff at the 95% confidence level (**Tables XXX-XXXI**).

Table XXX: Burden of Disease for Worst-Case *Ascaris* Infection Risk for Agricultural Worker Soil Ingestion (1000 Simulations)			
Model Description	**Control Points**	**Median (95% Conf. Interval) Burden of Disease Exponential Dose-Response (DALYs per person per year)**	**Median (95% Conf. Interval) Burden of Disease Beta-Poisson Dose-Response (DALYs per person per year)**
Ingestion of soil fertilized with compost containing ova concentrations modeled for eight-month old vermicompost, 126 days of exposure	Compost/Soil Ratio 100%	$1.32*10^{-1}$ ($1.32*10^{-1}$, $1.32*10^{-1}$)	$8.06*10^{-2}$ ($7.21*10^{-2}$, $9.08*10^{-2}$)
	Compost/Soil Ratio 50%	$1.31*10^{-1}$ ($1.30*10^{-1}$, $1.31*10^{-1}$)	$4.84*10^{-2}$ ($4.04*10^{-2}$, $5.67*10^{-2}$)
	Compost/Soil Ratio 10%	$8.85*10^{-2}$ ($7.64*10^{-2}$, $9.81*10^{-2}$)	$3.96*10^{-3}$ ($2.01*10^{-3}$, $6.83*10^{-3}$)

Table XXXI: Burden of Disease for Best-Case *Ascaris* Infection Risk for Agricultural Worker Soil Ingestion (1000 Simulations)			
Model Description	**Control Points**	**Median (95% Conf. Interval) Burden of Disease Exponential Dose-Response (DALYs per person per year)**	**Median (95% Conf. Interval) Burden of Disease Beta-Poisson Dose-Response (DALYs per person per year)**
Ingestion of soil fertilized with compost containing ova concentrations modeled for three-month old vermicompost, 126 days of possible exposure	Compost/Soil Ratio 100%	$1.19*10^{-1}$ ($1.11*10^{--1}$, $1.26*10^{-1}$)	$1.77*10^{-2}$ ($1.21*10^{-2}$, $2.59*10^{-2}$)
	Compost/Soil Ratio 50%	$8.96*10^{-2}$ ($7.80*10^{-2}$, $1.03*10^{-1}$)	$4.23*10^{-3}$ ($1.76*10^{-3}$, $7.90*10^{-3}$)
	Compost/Soil Ratio 10%	$1.98*10^{-2}$ ($1.35*10^{-2}$, $2.74*10^{-2}$)	$6.05*10^{-7}$ ($3.44*10^{-7}$, $1.10*10^{-6}$)

To define a measure for adequately sanitized composts, the maximum concentration of viable *Ascaris* ova in vermicompost that would yield an acceptable burden of disease (below 10^{-4} DALYs per person per year) for agricultural workers ingesting soil at sites of vermicompost application was modeled. A model including the control point of dilution of vermicompost in soil at the soil surface to 10% was used to estimate the maximum allowable viable *Ascaris* ova concentration in vermicompost, and was analyzed using the exponential and beta-Poisson dose-response functions. The maximum allowable viable ova concentration was calculated to be about 0.1 viable ova per gram of compost when using the exponential dose-response function. When the beta-Poisson dose-response function was used, the maximum allowable viable ova concentration was calculated to be about 1.02 viable ova per gram of compost (**Figure 13**). Both of these values were above the hypothetical lower limit of detection of 0.25 ova per gram TS (0.075 ova per gram compost) [12].

Figure 13: Burden of Disease by Ova Concentration for Ingestion of Soil by Agricultural Workers



Risk Scenario 3: Soil Ingestion by Children at Parks with Groundcover Fertilized with Vermicompost

For the risk scenario involving ingestion of soil by children aged 5-9 visiting parks fertilized with vermicompost, the effects on exposure of the modeled ova concentrations for the tested compost, as well as the dilution ratio of vermicompost in soil at the soil surface were analyzed.

Estimated median daily exposures to viable *Ascaris* ova from application of the tested vermicomposts ranged from 0.33 ova per day (3-month old, 18-month old vermicomposts) to 1.08 ova per day (8-month old vermicompost). The estimated median exposures from the tested composts were between 3 and 17 times greater than the estimated median exposure from application of a compost meeting the EPA cutoff for class A biosolids (0.25 ova per gram TS) (**Table XXXII**). The estimated median daily exposures for this scenario are higher than those for consumption of produce fertilized with vermicompost or ingestion of soil by workers laboring on fields fertilized with vermicompost.

Dilution of vermicompost at the surface of the soil decreases daily exposures proportionately to the ratio to which the vermicompost is diluted (**Table XXXIII**).

Table XXXII: Effect of Viable Ova Concentration in Vermicompost on Exposure in Risk Model for Risk Model for Child Soil Ingestion			
Variable Value	**Mean** ***Median*** **(99% CI)** **Viable Ova Ingested/Day**	**Relative Effect Compared to Reference Value**	**Interpretation**
EPA Cutoff of 0.25 viable ova/g TS	Daily Exposure: 0.247 *0.105* (0, 2.87)	Reference Value	Conservative estimate of exposure with *Ascaris* ova present at the limit of detection
San Roque 3-Month Vermicompost	Daily Exposure: 1.81 *0.33* (0, 34.54)	Mean exposure increased ~7.5 times Median exposure increased ~3 times 99.5th percentile exposure increased ~12 times	Conservative estimate of exposure with *Ascaris* ova present at levels modeled for 3-month vermicompost
San Roque 8-Month Vermicompost	Daily Exposure: 8.41 *1.88* (0, 161.75)	Mean exposure increased ~34 times Median exposure increased ~17 times 99.5th percentile exposure increased ~56.5 times	Conservative estimate of exposure with *Ascaris* ova present at levels modeled for 8-month vermicompost
San Roque 13-Month Vermicompost	Daily Exposure: 2.14 *0.40* (0, 40.42)	Mean exposure increased ~8.5 times Median exposure increased ~4 times 99.5th percentile exposure increased ~14 times	Conservative estimate of exposure with *Ascaris* ova present at levels modeled for 13-month vermicompost
Villa Mercurio 6-Month Vermicompost	Daily Exposure: 2.69 *0.45* (0, 56.75)	Mean exposure increased ~11 times Median exposure increased ~4.5 times 99.5th percentile exposure increased ~20 times	Conservative estimate of exposure with *Ascaris* ova present at levels modeled for 6-month vermicompost
Villa Mercurio 18-Month Vermicompost	Daily Exposure: 3.19 *0.33* (0, 62.51)	Mean exposure increased ~13 times Median exposure increased ~3 times 99.5th percentile exposure increased ~22 times	Conservative estimate of exposure with *Ascaris* ova at levels modeled for 18-month vermicompost

Table XXXIII: Effect of Dilution of Vermicompost at Surface of Soil on Exposure in Risk Model for Child Soil Ingestion			
Variable Value	**Mean** ***Median*** **(99% CI)** **Viable Ova Ingested/Day**	**Relative Effect Compared to Reference Value**	**Interpretation**
No Mixing, **Ratio = 1**	Daily Exposure: 8.41 *1.88* (0, 161.75)	Reference Value	Most conservative estimate of exposure, *Ascaris* ova present at levels modeled for 8-month vermicompost. Assumes that undiluted compost is present at surface of soil.
Even Mixing, **Ratio = 0.5**	Daily Exposure: 4.06 *0.94* (0, 71.69)	Daily exposure halved	Estimate of exposure with 50% mixing of compost with soil, *Ascaris* ova present at levels modeled for 8-month vermicompost. Represents application technique in which mixing or covering of compost with soil results in *Ascaris* ova concentrations at the surface half those found in the compost
Thorough mixing, **Ratio = 0.1**	Daily Exposure: 0.84 *0.19* (0, 16.68)	Daily exposure reduced by a factor of 10.	Estimate of exposure with 50% mixing of compost with soil, *Ascaris* ova present at levels modeled for 8-month vermicompost. Represents application technique in which mixing or covering of compost with soil results in *Ascaris* ova concentrations at the surface one tenth those found in the compost

The choice of dose-response model strongly influences estimates of daily risks of *Ascaris* infection for children visiting and ingesting soil from parks fertilized with vermicompost. Median daily infection risks estimated by the beta-Poisson dose-response model were roughly 9 times lower than those estimated by the exponential dose-response model. However, because exposure and daily risk of infection were so high with the viable ova concentrations modeled for the 8-month old vermicompost and no dilution of vermicompost at the surface of the soil, annual *Ascaris* infection risks remained at 100% regardless of the dose-response model used and the proximity of a child's home to the park (recall that children living nearby are assumed to visit the parks 3 days per week, while children living further away are assumed to visit 1 day per month on average, with a range of 0-4 visits per month) (**Table XXXIV**).

Table XXXIV: Effect of Dose-Response Model on Estimated *Ascaris* Infection Risk for Children Ingesting Soil at Parks Fertilized with Vermicompost

Variable Value	Mean *Median* 99% CI % Infection Risk	Relative Effect Compared to Reference Value	Interpretation
Exponential Dose-Response Model **Children living near parks (Near) have 12 days of exposure per month, children living further away (Far) have 0-4 days of exposure per month with an average of 1**	Daily Risk: 67.11 *83.92* (0, 100) Annual Risk (Near): 100 *100* (100, 100) Annual Risk (Far): 100 *100* (100, 100)	Reference Value	Conservative estimate of exposure (no soil mixing), *Ascaris* ova present at levels modeled for 8 month vermicompost. Exposure is Poisson distributed, single ova ingested causes infection 100% of time
Beta-Poisson Dose-Response Model **Children living near parks (Near) have 12 days of exposure per month, children living further away (Far) have 0-4 days of exposure per month with an average of 1**	Daily Risk: 11.77 *9.78* (0, 40.91) Annual Risk (Near): 100 *100* (100, 100) Annual Risk (Far): 100 *100* (100, 100)	Daily risks reduced by a factor of ~6-9 Annual risks unchanged because of high exposure (Effect size will vary as exposure changes, as dose-response functions have different slopes)	Conservative estimate of exposure (no soil mixing), *Ascaris* ova present at levels modeled for 8 month vermicompost. Estimated from Epidemiologic data among children aged 5-15 in Mexico. May not be applicable to other settings and age groups.

The best-case and worst-case *Ascaris* infection risks for children visiting parks fertilized with vermicompost were modeled using the ova concentrations estimated for the 3-month old vermicompost and the 8-month old vermicompost respectively. The best-case and worst-case risk models were stratified by proximity of children's houses to the park (children living nearby visit 3 days per week, children living further away visit 0-4 times per month), by dose-response model, and by the ratio of dilution of vermicompost in soil at the surface.

The median annual risks of *Ascaris* infection estimated by the worst-case model ranged from 99.70% to 100% for children living near parks and from 15.35% to 100% for children living further away from parks, with corresponding median daily risks of 1.71% to 86.36% (**Table XXXV**). Median annual risks estimated by the best-case model ranged from 82.70% to 100% for children living near parks and from 3.76% to 99.28% for children living further away from parks, with corresponding median daily risks of 0.31% to 29.58% (**Table XXXVI**). The lower limits of the 99% confidence intervals for burden of disease among children living distantly from the parks were often far below the median and upper bound. This variability is due to the small, semi-randomized number of visits (and therefore days of exposure) assumed in the risk model for children living farther from parks.

Table XXXV: Worst-Case *Ascaris* Infection Risk Distributions for Child Soil Ingestion (1000 Simulations)					
Model Description	**Control Points**	**Median (99% Conf. Interval) Daily Risk (%), Exponential Dose-Response**	**Median (99% Conf. Interval) Annual Risk (%), Exponential Dose-Response**	**Median (99% Conf. Interval) Daily Risk (%), Beta-Poisson Dose-Response**	**Median (99% Conf. Interval) Annual Risk (%), Beta-Poisson Dose-Response**
Ingestion of soil by children at parks fertilized with material containing the distribution of ova concentrations modeled for eight-month old vermicompost **Children living near parks (Near) have 12 days of exposure per month, children living further away (Far) have 0-4 days of exposure per month with an average of 1**	Compost/Soil Ratio 100%	86.36 (0.04, 100)	Near 100 (100, 100) Far 100 (100, 100)	10.36 (0, 39.92)	Near 100 (100, 100) Far 100 (100, 100)
	Compost/Soil Ratio 50%	63.03 (0.02, 100)	Near 100 (100, 100) Far 100 (12.52, 100)	6.45 (0, 35.53)	Near 99.77 (98.70, 99.98) Far 34.88 (2.13, 72.27)
	Compost/Soil Ratio 10%	18.09 (0, 100)	Near 100 (100, 100) Far 91.32 (2.67, 100)	1.71 (0, 24.12)	Near 99.70 (98.70, 99.95) Far 15.35 (0.42, 45.83)

Table XXXVI: Best-Case *Ascaris* Infection Risk Distributions for Child Soil Ingestion (1000 Simulations)					
Model Description	**Control Points**	**Median (99% Conf. Interval) Daily Risk (%), Exponential Dose-Response**	**Median (99% Conf. Interval) Annual Risk (%), Exponential Dose-Response**	**Median (99% Conf. Interval) Daily Risk (%), Beta-Poisson Dose-Response**	**Median (99% Conf. Interval) Annual Risk (%), Beta-Poisson Dose-Response**
Ingestion of soil by children at parks fertilized with material containing the distribution of ova concentrations modeled for three-month old vermicompost **Children living near parks (Near) have 12 days of exposure per month, children living further away (Far) have 0-4 days of exposure per month with an average of 1**	Compost/Soil Ratio 100%	29.58 (0, 100)	Near 100 (100, 100) Far 99.28 (3.34, 100)	2.81 (0, 31.28)	Near 99.98 (98.87, 100) Far 21.20 (8.66, 58.09)
	Compost/Soil Ratio 50%	16.14 (0, 100)	Near 100 (100, 100) Far 90.54 (5.44, 100)	1.51 (0, 24.59)	Near 99.64 (98.52, 99.93) Far 13.94 (0.13, 50.86)
	Compost/Soil Ratio 10%	3.42 (0, 95.27)	Near 100 (100, 100) Far 37.71 (0.46, 99.89)	0.31 (0, 14.04)	Near 82.70 (69.53, 91.93) Far 3.76 (0.12, 23.26)

The burden of disease for the best and worst-case scenarios was calculated according to the method adapted from Bundy, Chan *et al* [96]. None of the scenarios resulted in a median burden at or below the WHO proposed cutoff of 10^{-4} DALYs per person per year for an acceptable burden of disease. The estimated burden of disease for children living near the parks never fell below 10^{-1} DALYs per person per year. The median estimated burden of disease for children living more distantly from the parks but visiting 0-4 times per month ranged from $9.05*10^{-4}$ (best-case scenario) to $2.34*10^{-1}$ (worst-case scenario) DALYs per person per year (**Tables XXXVII-XXXVIII**).

Table XXXVII: Burden of Disease for Worst-Case *Ascaris* Infection Risk for Child Soil Ingestion (1000 Simulations)			
Model Description	**Control Points**	**Median (95% Conf. Interval) Burden of Disease Exponential Dose-Response (DALYs per person per year)**	**Median (95% Conf. Interval) Burden of Disease Beta-Poisson Dose-Response (DALYs per person per year)**
Ingestion of soil fertilized with compost containing ova concentrations modeled for eight-month old vermicompost, 126 days of exposure **Children living near parks (Near) have 12 days of exposure per month, children living further away (Far) have 0-4 days of exposure per month with an average of 1**	Compost/Soil Ratio 100%	Near $2.34*10^{-1}$ $(2.34*10^{-1}, 2.34*10^{-1})$ Far $2.34*10^{-1}$ $(2.34*10^{-1}, 2.34*10^{-1})$	Near $2.34*10^{-1}$ $(2.34*10^{-1}, 2.34*10^{-1})$ Far $2.34*10^{-1}$ $(2.34*10^{-1}, 2.34*10^{-1})$
	Compost/Soil Ratio 50%	Near $2.34*10^{-1}$ $(2.34*10^{-1}, 2.34*10^{-1})$ Far $2.34*10^{-1}$ $(1.32*10^{-2}, 2.34*10^{-1})$	Near $2.33*10^{-1}$ $(2.30*10^{-1}, 2.34*10^{-1})$ Far $7.54*10^{-2}$ $(2.20*10^{-2}, 1.19*10^{-1})$
	Compost/Soil Ratio 10%	Near $2.34*10^{-1}$ $(2.34*10^{-1}, 2.34*10^{-1})$ Far $2.10*10^{-1}$ $(8.03*10^{-2}, 2.33*10^{-1})$	Near $2.33*10^{-1}$ $(2.30*10^{-1}, 2.34*10^{-1})$ Far $2.56*10^{-2}$ $(7.61*10^{-4}, 6.32*10^{-2})$

Table XXXVIII: Burden of Disease for Best-Case *Ascaris* Infection Risk for Child Soil Ingestion (1000 Simulations)			
Model Description	**Control Points**	**Median (95% Conf. Interval) Burden of Disease Exponential Dose-Response (DALYs per person per year)**	**Median (95% Conf. Interval) Burden of Disease Beta-Poisson Dose-Response (DALYs per person per year)**
Ingestion of soil fertilized with compost containing ova concentrations modeled for three-month old vermicompost, 126 days of possible exposure **Children living near parks (Near) have 12 days of exposure per month, children living further away (Far) have 0-4 days of exposure per month with an average of 1**	Compost/Soil Ratio 100%	Near $2.34*10^{-1}$ $(2.34*10^{-1}, 2.34*10^{-1})$ Far $2.32*10^{-1}$ $(3.15*10^{-2}, 2.34*10^{-1})$	Near $2.34*10^{-1}$ $(2.34*10^{-1}, 2.34*10^{-1})$ Far $3.59*10^{-2}$ $(6.43*10^{-3}, 8.08*10^{-2})$
	Compost/Soil Ratio 50%	Near $2.34*10^{-1}$ $(2.34*10^{-1}, 2.34*10^{-1})$ Far $2.02*10^{-1}$ $(2.04*10^{-2}, 2.34*10^{-1})$	Near $2.33*10^{-1}$ $(2.32*10^{-1}, 2.33*10^{-1})$ Far $2.23*10^{-2}$ $(1.20*10^{-3}, 5.62*10^{-2})$
	Compost/Soil Ratio 10%	Near $2.34*10^{-1}$ $(2.34*10^{-1}, 2.34*10^{-1})$ Far $6.84*10^{-2}$ $(1.09*10^{-5}, 2.30*10^{-1})$	Near $1.88*10^{-1}$ $(1.70*10^{-1}, 2.01*10^{-1})$ Far $9.05*10^{-4}$ $(3.53*10^{-6}, 1.98*10^{-2})$

To define a measure for adequately sanitized vermicomposts for application to public greenspace, the relationship of viable ova concentration in the composts to the burden of disease among children visiting the sites of application was modeled. The model was constructed using the control point of dilution of vermicompost at the soil surface to 10%, and was analyzed separately for children living nearby the site of application and for children living more distantly but occasionally visiting the site of application and stratified by dose-response function.

The maximum allowable viable *Ascaris* ova concentration in vermicompost applied to public greenspace when considering risks to children living near the site of application was calculated to be about $5.6*10^{-4}$ viable ova per gram when using the exponential dose-response model, and about $6.4*10^{-3}$ viable ova per gram when using the beta-Poisson dose-response model (**Figure 14**). When considering risks to children living further from the site of application (and visiting less frequently) the calculated risk/burden of disease was highly variable, but approximate maximum acceptable viable ova concentrations in vermicompost were $1.7*10^{-2}$ viable ova per gram when using the exponential dose-response model and 0.126 viable ova per gram when using the beta-Poisson dose-response model (**Figure 15**). Taking into consideration a lower limit of detection of *Ascaris* ova in biosolids of 0.25 ova per gram TS [12] (or roughly 0.075 ova per gram vermicompost for the composts examined in this study), the only scenario with a maximum allowable viable ova concentration within the detectable range is for children living distantly from the site of application of vermicompost, using the beta-Poisson dose-response model to estimate risk of infection.

Figure 14: Burden of Disease by Ova Concentration for Ingestion of Soil by Children Living Near Public Parks Fertilized with Vermicompost

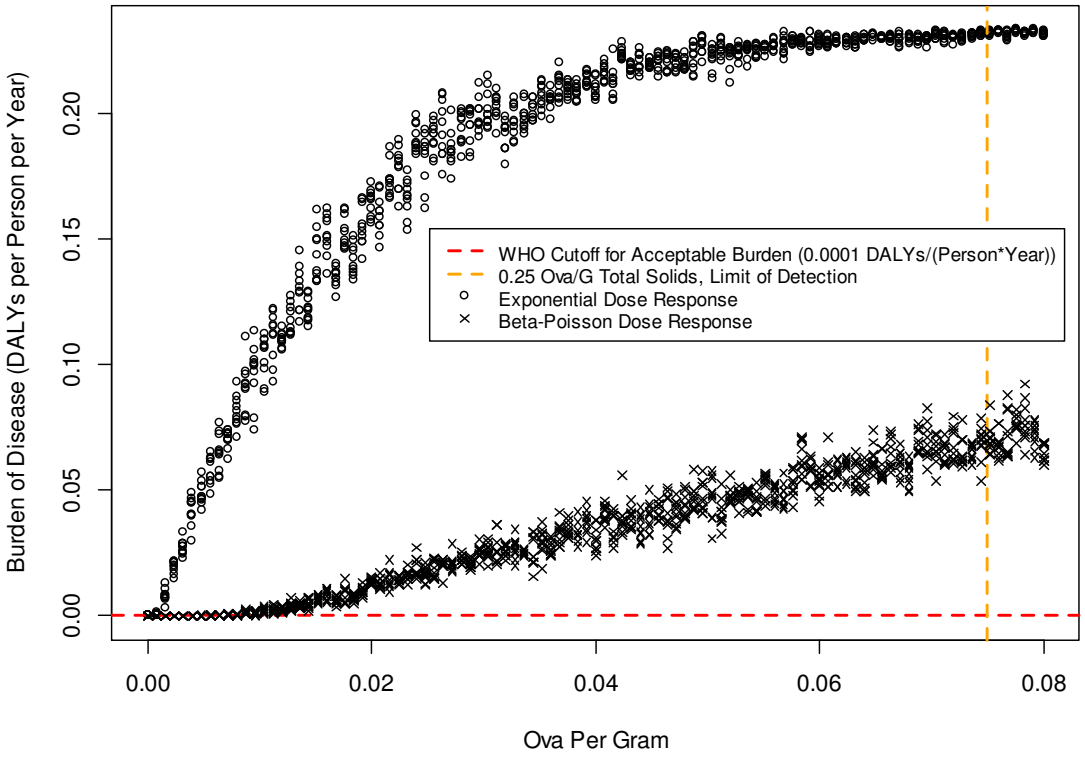
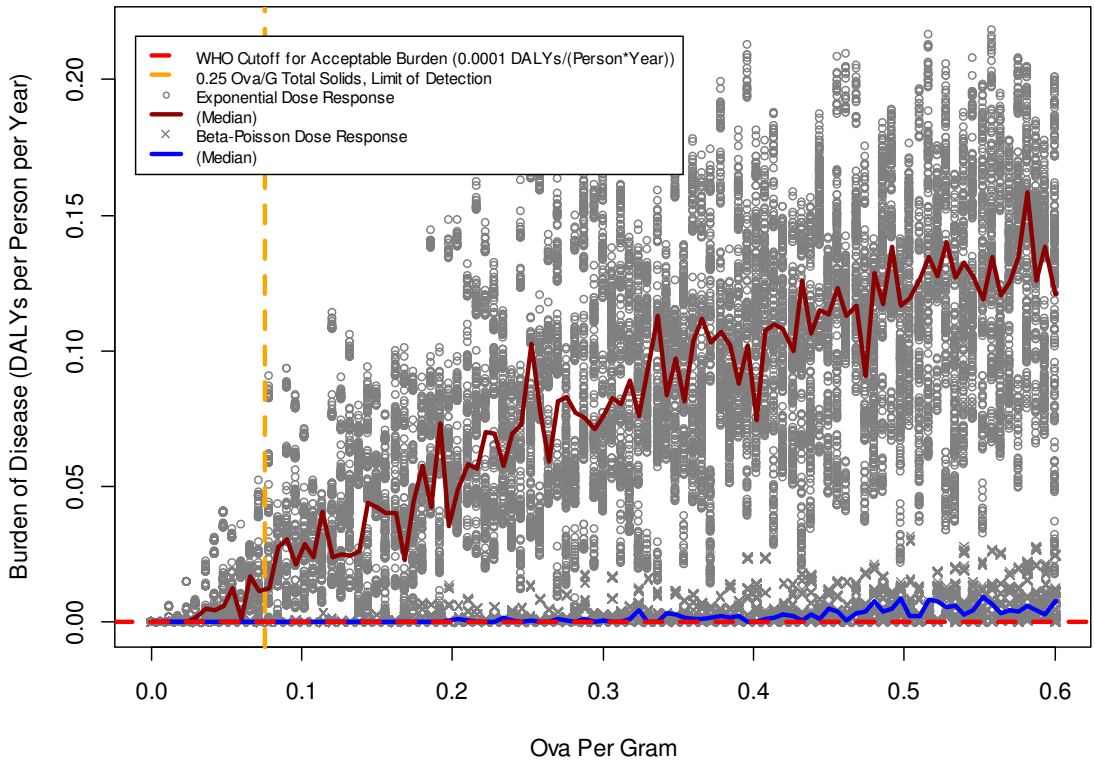


Figure 15: Burden of Disease by Ova Concentration for Ingestion of Soil by Children Living Further from Public Parks Fertilized with Vermicompost



Modeled Accumulation of *Ascaris* Ova in Soil with Repeated Application of Vermicompost

Due to the long-term viability of *Ascaris* ova in soils, risks to consumers of produce fertilized with Ecosan composts, workers at sites fertilized with Ecosan composts, and children playing on sites fertilized with Ecosan composts are likely to increase over time if composts containing *Ascaris* ova are repeatedly applied to the same site. To demonstrate the extent to which exposure may increase, **Figures 16-19** display modeled accumulation of viable ova in soils with 4 times annual, biannual, annual, and biennial application of compost having an *Ascaris* ova content similar to that modeled for the three month vermicompost, assuming the time to 90% inactivation of the ova is normally distributed with a mean of 625 days and a standard deviation of 150 days [101], and that ova are not removed by other processes.

Figure 16: Projected Accumulation of Viable *Ascaris* Ova in Soil with 4 Times Annual Application of Compost

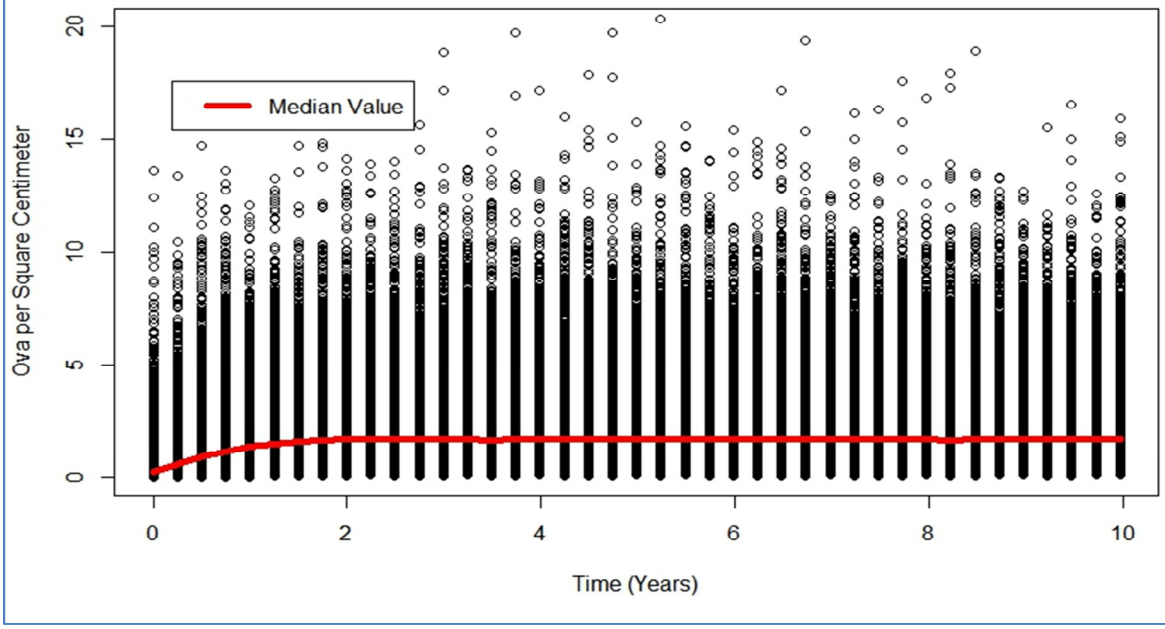


Figure 17: Projected Accumulation of Viable *Ascaris* Ova in Soil with Biannual Application of Compost

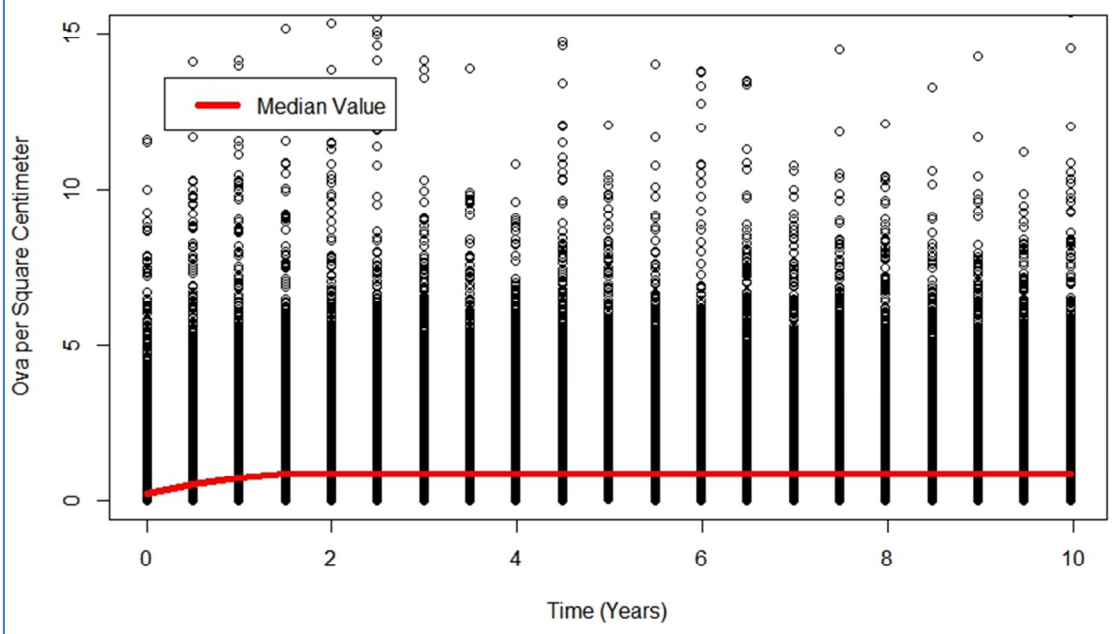


Figure 18: Projected Accumulation of Viable *Ascaris* Ova in Soil with Annual Application of Compost

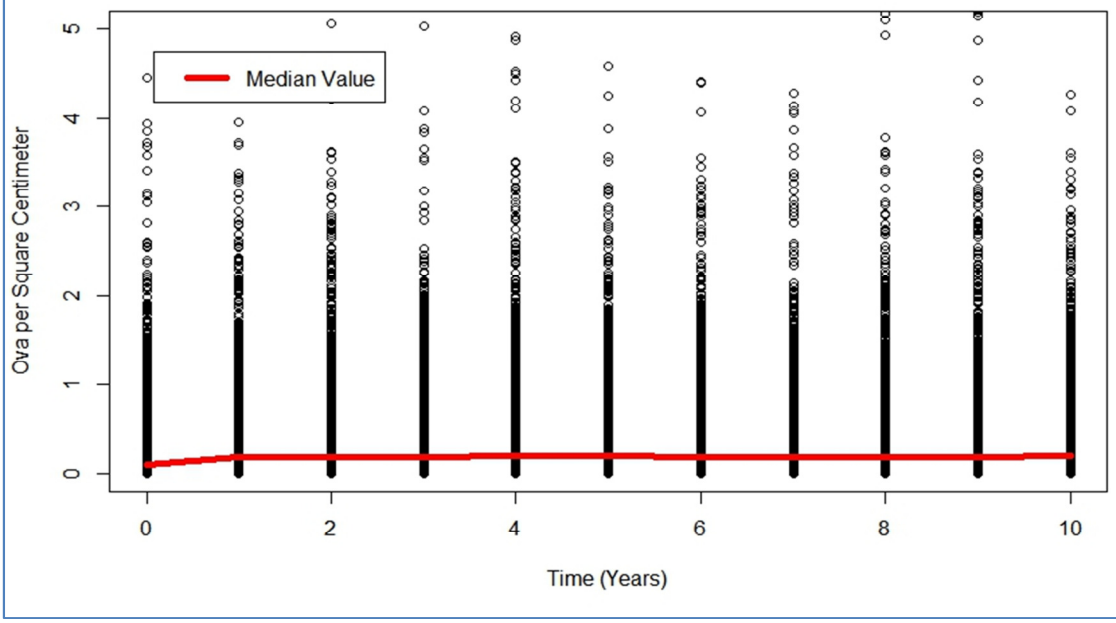
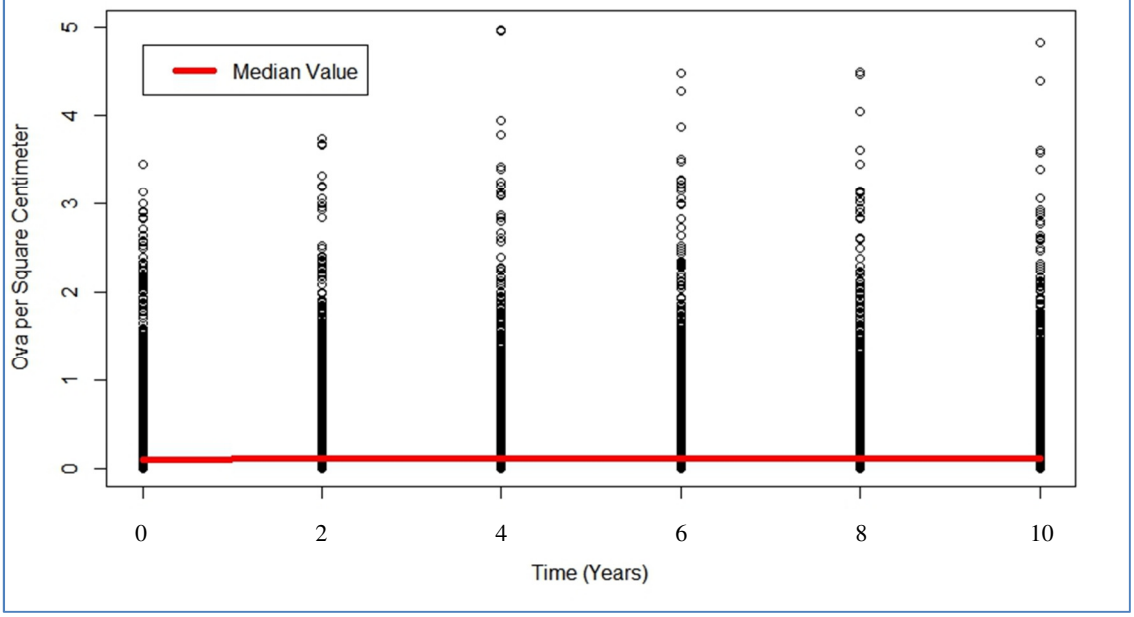


Figure 19: Projected Accumulation of Viable *Ascaris* Ova in Soil with Biennial Application of Compost



As shown in **Figure 16**, the median ova concentration at the soil surface was projected to increase from 0.196 to about 1.72 viable ova/cm^2 (an 8.78 fold increase) within the first two years of four times yearly repeated application of Ecosan compost with ova concentrations modeled for the three month vermicompost. Such a scenario might be encountered in the fertilization of grass or other groundcover. **Figure 17** shows a rise in median ova concentrations from 0.122 to about 0.469 viable ova/cm^2 (a 3.81 fold increase) after two years of biannual application of vermicompost, as might be found in cultivation of food crops. **Figure 18** shows a rise in median ova concentrations from 0.094 to 0.192 viable ova/cm^2 (a 2.03 fold increase) if the frequency of application of vermicompost is restricted to once every year. **Figure 19** shows a rise in median ova concentrations from 0.10 to 0.11 viable ova/cm^2 (a 1.1 fold increase) for a scenario in which the frequency of application of vermicompost is restricted to once every 2 years. Depending on the frequency of application of vermicompost, these observations may further reduce the maximum allowable ova concentrations derived from the above risk models by a factor of 1.1 to 8.8.

Discussion

The goal of this study was to assess the risks to human health from *Ascaris* infections caused by reuse of vermicomposted excreta in horticultural settings. The intended outcomes were twofold: to estimate risks found in the specific sanitation system examined in El Alto, Bolivia for the sake of informing local policy and practice, as well as to extrapolate observations from the El Alto system to comment on the suitability of vermicomposting as a sanitization strategy and the safety of reutilization of biosolids in general.

The results of this study indicate the presence of unsafe levels of viable *Ascaris* ova in the composts examined. Movement of *Ascaris* larvae within or freshly emerged from their eggs, the surest sign of viability, was not observed in the processed samples, with the exception of a single motile larva in the 18-month compost. However, morphologically intact ova containing fully matured, infective larvae were observed. Mean viable *Ascaris* ova concentrations in each of the composts examined were greater than the WHO recommended limit of 1 ova/g total solids (TS). Mean viable ova concentrations were 1.02 ova/g TS, 1.18 ova/g TS, 7.60 ova/g TS, 1.43 ova/g TS, and 1.91 ova/g TS, in composts processed for 3, 6, 8, 13, and 18 months respectively. Bayesian models estimating the rate of decay of ova in the vermicomposts returned 95% confidence intervals for 90% inactivation time ranging from 1.06 months to 39,100 months, with median times of 9.8 to 29.28 months, indicating that the data contain no evidence of rapid inactivation of *Ascaris* ova by vermicomposting.

The risk models constructed in this study indicate that reuse of the vermicomposted excreta from the examined ecological sanitation system would likely result in unacceptably high burdens of disease from *Ascaris* infection among

consumers of raw produce grown with vermicompost, agricultural workers laboring on fields fertilized with vermicompost, and children aged 5-9 playing in public parks where groundcover has been fertilized with vermicompost. Worst and best-case assumption estimates for the median burden of disease for individuals exposed through consumption of raw carrots fertilized with the composts were $1.59*10^{-1}$ and $1.64*10^{-5}$ DALYs per person per year, respectively. For consumption of raw spinach, worst and best-case scenario estimates of median burden of disease were $1.59*10^{-1}$ and $2.41*10^{-3}$ DALYs per person per year. The median burden of disease faced by agricultural workers at sites fertilized with vermicomposts was estimated to be $1.32*10^{-1}$ DALYs per person per year under the worst-case assumptions, and $6.05*10^{-7}$ DALYs per person per year under the best-case assumptions. The worst and best-case median burdens of disease among children aged 5-9 years ingesting soil during play in public parks fertilized with vermicompost were estimated to be $2.34*10^{-1}$ and $1.88*10^{-1}$ DALYs per person per year for children living near the parks, and $2.34*10^{-1}$ and $9.05*10^{-4}$ DALYs per person per year for children living further from the parks[1].

In many of the modeled scenarios, the concentration of viable ova required for an acceptable burden of disease of 10^{-4} DALYs per person per year [14] was well below the limit of detection of the EPA method for helminth ova recovery (0.25 ova/g TS, or about 0.075 ova/g for the vermicomposts examined) [12]. For consumption of

1. The differences between the worst and best-case models for each scenario included use of the highest and lowest mean viable *Ascaris* ova concentrations observed in the sampled vermicomposts for the worst and best-case models respectively, no dilution of vermicompost at soil surface for the worst-case models or dilution to 10% in soil for the best-case models, and use of the single hit exponential dose-response function in the worst-case models and the beta-Poisson dose-response function in the best-case models. Additionally, for the scenario of consumption of raw produce fertilized with vermicompost, the worst-case models included 365 days of exposure while the best-case models included only 56 days of exposure, as well as a tap water rinse of produce prior to consumption.

raw carrots fertilized with vermicompost, acceptable risks were obtained at no more than 0.21 viable ova/g when using the beta-Poisson dose-response model proposed by Navarro *et al.* [93], and no more than 0.02 viable ova/g when using a single hit exponential model. For the consumption of raw spinach, these values shifted to a maximum acceptable ova concentration of 0.026 viable ova/g with the beta-Poisson dose-response model, and no more than 0.003 viable ova/g with the single hit exponential model[1]. When modeling risks to agricultural workers on sites fertilized with the vermicomposts, the maximum allowable ova concentration was estimated to be 1.02 viable ova/g when using the beta-Poisson model and 0.1 viable ova/g when using the single hit exponential model. When modeling risks to children aged 5-9 years living near and playing in parks fertilized with vermicompost, the maximum allowable ova concentration was estimated to be $6.4*10^{-3}$ viable ova/g when using the beta-Poisson dose-response model and $5.6*10^{-4}$ viable ova/g when using the exponential dose-response model. For children living at greater distances from the parks, maximum allowable ova concentrations were calculated to be 0.126 viable ova/g and 0.017 viable ova/g with the beta-Poisson and exponential dose-response models, respectively.

An examination of the likely accumulation of ova with repeated application of composts to soils demonstrated that *Ascaris* ova concentrations in soil, which directly correlate to daily exposures in the risk models for each scenario, were likely to increase by a factor of between 1.1 to 8.8 over a span of two years depending on the frequency of application of vermicompost. Therefore, if, for instance, growers wanted

1. Maximum allowable ova concentrations for growing produce that may be consumed raw were calculated under the assumption that it would be most appropriate to use 365 days of exposure per year, since long-term sustainability of an ecological sanitation system may depend on being able to sell composted excreta to growers year-round.

to apply vermicompost to the same area of land twice per year, the maximum allowable concentrations of viable *Ascaris* ova for each risk scenario would be decreased by a factor of 3.81. This would lower the maximum allowable concentration of viable ova below the limit of detection for every risk scenario except for risks to agricultural workers, and then only if the beta-Poisson dose-response model is used in place of the exponential single hit dose-response model.

There are many limitations to this study which should be considered when interpreting its results. The degree to which the results of this study are informative with respect to the inactivation of *Ascaris* ova by vermicomposting is limited because sampling was done in a cross-sectional manner, taking data from several different composts that had been started at different times, rather than in a prospective manner, in which the viability of ova in a cohort of composts would be monitored over time. Additionally, while the single motile larva intra-ovum observed in the 18 month-old compost would seem to imply that vermicomposting does not result in 100% inactivation of *Ascaris* ova within 18 months, the significance of this observation is uncertain due to the lack of fencing around the Villa Mercurio composting site. The motile larvum may have been a survivor from the initial cohort of ova within the compost, or it may have been deposited or transferred by one of the many animals observed to be kept in the area.

Due to limited time and materials for the laboratory components of the study, as well as the time-consuming nature of the protocols for quantifying viable *Ascaris* ova, sample sizes were very small, ranging from a minimum of three samples for the 6 month and 18 month composts sampled at Villa Mercurio to a maximum of nine samples for the 8 month old compost at San Roque. Although Bayesian models were

constructed to provide simulated ova concentrations for risk assessment, the small numbers of observations may have contributed to very long probability tails in the simulated distributions, possibly leading to some overestimates of exposure and risk.

Reported methods for identifying and quantifying viable *Ascaris* ova are variable. Some authors have reported all ova lacking evident necrotic morphologies or obvious damage as viable [28]. Some have based viability status on the exclusion of dyes such as methylene blue [47]. Others have quantified any ova with observable ordered internal structures as viable [49]. The most common approach, and the one taken by this study, is to classify all ova containing a fully developed, non-damaged larva as viable [26, 40]. However, this approach may be prone to misclassification of inactivated, yet morphologically pristine, embryonated ova as viable.

Data supporting many of the values used for variables in the risk models were not available or were based on single observations. The application rate of the composts was used as a single value with no estimates of uncertainty and was based on email communication with one individual involved in the vermicomposting effort in Bolivia. The dilution factors for helminth ova in compost at the surface of treated soils were arbitrarily selected to provide some effect of safer and less-safe techniques of biosolid application within the models, but were not based on empirical data or recommendations. The functions describing transfer of ova from soil to the surfaces of food crops were taken from single data points published by Jimenez *et al.* [111] due to lack of measures of variability in the publication. When contacted, the author was unfortunately unable to access the study data due to a recent international move. The survival function ultimately chosen for analyses of infection risks from ingesting raw produce was a time-independent proportion taken from two observations published in

separate papers [102, 111] without measures of variability. While some published data was available to estimate the daily consumption of vegetables by Bolivians, the amounts of raw carrots and raw spinach consumed were arbitrarily assigned to account for ¼ of the total vegetable consumption apiece. Neither of the dose-response curves available for *Ascaris* is based on clinical data, the single hit exponential model being a worst-case assumption, and the beta-Poisson model being derived from epidemiological data for 5-15 year old children in the Mezquital valley of Mexico through assumptions and methods not easily discernible in the original publication [93], and here extrapolated to an all-ages population in a different geographical region and climate. The rate of soil ingestion for adult agricultural workers is not strongly supported by empirical data, and so a relatively uncertain uniform distribution was used [106]. No effects of personal protective equipment (PPE) or daily changes in labor activities were incorporated into the risk model for adult agricultural laborers. The behavior of children visiting parks was based on the subjective recollections of an individual who had lived in Bolivia rather than on empirical data. Finally, the estimates of daily soil ingestion distributions for children playing in public spaces were taken from data obtained in a different country [108] and assumed to be applicable in Bolivia.

The method used to calculate the burden of disease due to *Ascaris* infection is based on incomplete knowledge of the true impact of ascariasis on human health [96]. The model may estimate the burden of disease for children under 10, and does not take into account the effect of helminth infections as risk factors for other diseases. Furthermore, the method for calculating burden of disease is here applied in a different context than the calculations of global burden of disease attributable to ascariasis for

which it was originally developed [4, 95]. It is possible that the dynamics of *Ascaris* transmission due to application of contaminated vermicomposts to the growth of food crops or in public greenspace would not be the same as for the conditions under which the calculations to predict disease burden were derived. In other words, the relationship between prevalence of infection and the distribution of worm burdens may be different within the populations at risk from reuse of vermicomposts in horticulture than it would be for a population for which the primary means of transmission is through general environmental contamination and poor sanitation.

Despite the aforementioned limitations, this study has strengths that should encourage the use of its results in guiding current policies and identifying key areas for future research. Currently, there are very few published risk assessments (prominently [93] and [101]) addressing the transmission of *Ascaris* via application of biosolids, and as such this study presents key data for an understudied, but important, field. On a similar note, the state of published knowledge on the efficacy of vermicomposting for the inactivation of helminth ova is very ambiguous. By contributing data that can be used to judge vermicomposting as a sanitization strategy, this study provides another piece of evidence that may be used to shape effective ecological sanitation interventions. This study also has strengths in its modeling of risks for several groups likely to be affected by strategies incorporating the application of biosolids to horticultural lands and its unprecedented (within *Ascaris* biosolids application risk assessments) examination of the potential effects of accumulation of *Ascaris* ova. Finally, while there are reasons to question the extent to which the results of the models constructed in this study reflect real conditions, the models are unanimous and conclusive in demonstrating that reuse of the examined vermicomposts in horticultural

applications would produce unacceptably high health risks in all but the best-case scenarios.

While the current study was not properly structured to prove or disprove the ability of vermicomposting to inactivate *Ascaris* ova, the presence of a definitely viable, motile larva in the eighteen month compost, as well as the general presence of apparently viable ova in all examined composts, regardless of processing time, does not imply a dramatic effect of vermicomposting on *Ascaris* viability. The Bayesian models constructed for ova die-off confirm that the data does not support an assertion that vermicomposting rapidly inactivates *Ascaris*. While vermicomposting has been suggested to be a suitable option for sanitization and destruction of helminth ova by some studies and publications [56, 71-75], such a claim has been weakly supported in all cases. Rodriguez-Canche *et al.* observed concurrent inactivation of helminth ova in the absence of vermicomposting [72]. Vigueros *et al.* and Contreras-Ramos *et al.* reported 0 viable helminth ova after composting, but lacked helminth ova in the initial substrate [73, 74]. Cardoso-Vigueros *et al.* reported helminth ova inactivation in a process including high temperature composting of long duration prior to vermicomposting [71]. Edwards optimistically conflates the action of earthworms against free-living nematodes with the fate of resilient nematode ova passing through the earthworm digestive tract [56]. In contrast, the findings of Jones *et al.* who reported some protection of *Ascaris* ova from attacks by soil fungus after passage through the earthworm gut, and Bowman *et al.*, who reported no significant effect of vermicomposting on *Ascaris* viability in a prospective study, are in better agreement with the findings of the current study, and suffer to a lesser extent from confounding factors [79, 112].

The present study found that *Ascaris* infection risks to consumers ingesting raw spinach or carrots fertilized with vermicomposts, laborers doing work in fields fertilized with vermicomposts, and children playing in public spaces fertilized with vermicomposts were likely to exceed the WHO-proposed acceptable burden of disease due to application of biosolids in horticulture of 10^{-4} DALYs per person per year. In 25 out of 32 scenarios examined for consumption of raw produce, 6 out of 16 scenarios examined for workers on fields fertilized with the composts, and 18 out of 32 scenarios examined for children playing in public parks fertilized with the composts, mean annual risks of infection were greater than 50%. There are very few published risk assessments investigating *Ascaris* risk due to application of biosolids with which to compare these results. Two existing studies have drawn somewhat similar conclusions on the severity of the risk of *Ascaris* transmission due to application of biosolids. Navarro *et al.* [113] found an annual infection risk of 100% if raw spinach was consumed without any control barriers (i.e. washing produce prior to consumption), and noted that the annual risk of infection remained near 10-17% even if the ova content was reduced to 0.25 ova/g TS and a detergent wash removing 99% of ova from produce was implemented. Schönning *et al.* [101] modeled *Ascaris* infection risks from local reuse of stored feces in gardening applications, and found risk levels in excess of their acceptable limit of 10^{-4} probability of infection per person per year despite an annual incidence of ascariasis for the population in the model of only $2*10^{-5}$ per person per year. The elevated risks modeled for helminth infections associated with reuse of excreta in agriculture are also in general agreement with epidemiological evidence. Habbari *et al.* [114] observed a greater than twofold higher prevalence of *Ascaris* infection in Moroccan children exposed to wastewater irrigated

land in comparison to those who were not exposed. Corrales *et al.* [17], reported twofold higher odds of trichuris infection and fourfold higher odds of hookworm infection among El Salvadoran families with Ecosan latrines that used the composted excreta in their gardens than were reported for families that simply buried the excreta.

The findings of this study and the literature reviewed have important implications for sanitation policy. Most strikingly, the study found that application of biosolids to agricultural fields, work areas, or public spaces could result in unacceptably high risks of *Ascaris* transmission even if the concentration of viable *Ascaris* ova is lower than the limit of detection of 0.25 ova/g TS. Accordingly, these results recommend that, until the dose-response relation of exposure to viable *Ascaris* ova to infection is better understood, use of biosolids in horticulture be restricted from agricultural applications where food crops that may be eaten raw have a chance of contacting soil treated with biosolids, as well as from public spaces and areas of manual labor, unless the prevalence of *Ascaris* infection is known to be extremely low within the population contributing the biosolids. Furthermore, any allowed applications of biosolids should be restricted to a biennial basis in order to prevent increases in risk due to accumulation of viable ova in soils. Any produce that may be expected to come into contact with biosolids during production should be required to receive a thorough rinse with clean water prior to being brought to market in order to reduce the risk of *Ascaris* transmission.

Manual laborers in ecological sanitation systems, whether involved in collecting and transporting Ecosan composts or in applying those composts to soils, are likely to be at elevated risk for helminth infections. In recognition of this risk, appropriate personal protective equipment, such as gloves, surgical masks, boots, and

exterior clothing, should be provided along with proper training in safe methods for handling and application of biosolids. Personal protective equipment should be provided at the site of work with Ecosan composts, and should not leave the contaminated site so that helminth ova will not be borne to new areas on workers' clothing. In recognition of the risk inherent in their occupations, workers handling Ecosan composts should be given the option of receiving periodic antihelminthic medication to prevent development of chronic infections.

The literature review presented at the opening of this thesis noted that there is a lack of concrete evidence in published literature that vermicomposting may lead to the inactivation of helminth ova, especially *Ascaris* ova, which are the hardiest species of intestinal pathogen in the environment. The observations made in this study do not provide any new evidence supporting the suitability of vermicomposting for helminth inactivation, and may imply the opposite. Given that helminth ova are likely to represent the greatest health risk in sanitation schemes involving re-utilization of composted feces, the lack of evidence supporting vermicomposting as a means of destroying helminth ova should preclude the consideration of vermicomposting as the major sanitization step in any ecological sanitation system. Vermicomposting remains valuable as a process for improving the productive potential of Ecosan composts, but should be combined with more proven sanitization methods such as ammonia treatment at high pH and high temperature thermophilic composting.

Conclusion

In conclusion, this study found no evidence of accelerated inactivation of helminth ova by vermicomposting, and estimated very high risks to consumers of raw produce, agricultural workers laboring in fields, and children playing in public spaces fertilized with the examined vermicomposts. In all scenarios modeled, except the estimates for risks to agricultural workers, acceptable burdens of disease below 10^{-4} DALYs per person per year were achieved with viable ova concentrations at or above the reliable limit of detection of 0.25 ova/g TS only under the best-case assumptions, including use of the beta-Poisson dose-response model published by Navarro *et al*., the suitability of which is uncertain outside of their study setting and population [93].

The reliability of these results is limited by the small number of observations as well as gaps in data and assumptions made for the constructed risk models. Nonetheless, until more refined models and data are available, these findings should be taken seriously, and policies should be put into place restricting the application of biosolids from ground crops that have any likelihood of being consumed raw, to areas of manual agricultural labor, and from public spaces such as parks. Furthermore, vermicomposting should not be considered a viable sanitization strategy for Ecosan systems until rigorous empirical evidence supports its ability to inactivate helminth ova. Barring the event that such evidence is published, vermicomposting should be considered only as a step for improving the fertilization value of Ecosan composts, and should be combined with sanitization strategies of demonstrated potency, such as thermophilic composting and ammonia/high pH treatment.

Future directions for research to enhance or amend the findings of this study include generation of clinical dose-response data for *Ascaris* infection, generation of a

scale for estimating the intensity of health effects as worm burden increases, risk assessments incorporating the effect of worm burden into DALY calculations, quantitative data describing the inactivation of helminth ova on crop surfaces over time, more empirical estimates of adult soil ingestion rates during agricultural labor, quantitative data on the effect of different techniques of applying biosolids to soils on transfer of helminth ova to food crops, as well as helminth ova concentration at the soil surface, and quantitative prospective studies of pathogen survival in Ecosan vermicomposting conditions.

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