Does the use of tubular digesters to treat livestock waste lower the risk of infection from *Cryptosporidium parvum* and *Giardia lamblia*?

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ABSTRACT

Worldwide, high incidences of cryptosporidiosis and giardiasis are attributed to livestock waste. Quantitative microbial risk assessment can be used to estimate the risk of livestock related infections from *Cryptosporidium parvum* and *Giardia lamblia*. The objective of this paper was to assess the occupational and public health risks associated with management of raw and anaerobically digested livestock waste in two rural communities in Costa Rica based on fomite, soil and crop contamination and livestock waste management exposure pathways. Risks related to cattle waste were greater than swine waste due to cattle shedding more (oo)cysts. *Cryptosporidium parvum* also posed a greater risk than *Giardia lamblia* in all exposure pathways due to livestock shedding high loads of *Cryptosporidium parvum* oocysts and oocysts' lower inactivation rates during anaerobic digestion compared with *Giardia lamblia* cysts. The risk of infection from exposure to contaminated soil and crops was significantly lower for a community using tubular anaerobic digesters to treat livestock waste compared to a community where the untreated waste was applied to soil. The results indicate that treatment of livestock waste in small-scale tubular anaerobic digesters has the potential to significantly decrease the risk of infection below the World Health Organization's acceptable individual annual risk of infection (10^{-4}).

Key words | *Cryptosporidium parvum*, *Giardia lamblia*, livestock waste, risk assessment, tubular digesters

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INTRODUCTION

Around the world, food-borne and water-borne outbreaks have been caused by pathogens from livestock wastes. Runoff from land-applied livestock waste has been the main contributing factor to these outbreaks (Brooks *et al.* 2012; Dufour *et al.* 2012). The presence of pathogens, such as *Cryptosporidium* sp., *Giardia lamblia* (also referred to as *Giardia duodenalis*), *Ascaris lumbricoides*, *Entamoeba histolytica*, *Escherichia coli* and fecal coliforms, in raw vegetables sold in open markets in Costa Rica, Egypt and Nigeria has also been attributed to use of irrigation water contaminated by livestock waste (Monge & Chinchilla 1996; Damen *et al.* 2007; Eraky *et al.* 2014). Although there are a variety of zoonotic pathogens that cause illness to humans, two protozoan parasites, *Cryptosporidium parvum* and *Giardia lamblia*, and three bacteria, *Campylobacter jejuni*, *Salmonella* sp. and *E. coli* O157:H7 were identified by the World Health Organization (WHO) as the main zoonotic pathogens of concern (Dufour *et al.*

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2012). The reasons for this include: (1) disease from these pathogens occur in healthy humans and can result in serious illness and/or death; (2) these pathogens are distributed globally; (3) they are resistant to commonly used disinfection technologies, such as chlorination; (4) the livestock genotypes are closely related to human genotypes; and (5) water transmission is the main route of exposure (Dufour *et al.* 2012).

Livestock waste can contain high loads of these zoonotic pathogens, in particular the protozoan parasites, *Cryptosporidium parvum* and *Giardia lamblia*. *Cryptosporidium parvum* accounts for 23.7% of all reported worldwide waterborne outbreaks annually while *Giardia lamblia* infects about 4% (0.28 billion people) worldwide annually (Dufour *et al.* 2012). Researchers from different regions of the world have shown that mismanagement of swine and cattle manure has led to contaminated food and water which may have led to foodborne outbreaks (Monge & Chinchilla, 1996; Slifko *et al.* 2000; Tai-Lee 2002; Farzan *et al.* 2011; Feng *et al.* 2011; Siwila & Mwape 2012). In developing countries where the water and food distribution systems are lacking and contamination is common, the probability of exposure to these parasites increases. However, since surveillance systems for detecting outbreaks in poor communities in developing countries is uncommon, foodborne outbreaks from exposure to contaminated livestock waste is rarely reported.

The high infectivity at low doses of these parasites also increases their associated public health concerns. Based on an annual acceptable risk of 10^{-4}, the acceptable concentration of *Giardia lamblia* cyst in potable water is 6.75×10^{-6} cysts/L and 3.27×10^{-5} oocysts/L for *Cryptosporidium parvum* oocysts (Regli *et al.* 1991; Haas *et al.* 1996). Young, old and immunocompromised individuals are particularly susceptible to disease from infection with these pathogens (Haas *et al.* 1999). Symptoms from giardiasis and cryptosporidiosis include diarrhea or gastroenteritis and can be fatal for immunocompromised individuals (Dufour *et al.* 2012). Gao *et al.* (2015) carried out a disease burden analysis for infections from *Cryptosporidium* sp. and *Giardia* sp. originating from livestock waste for communities in China and Ghana. The authors found that infections from these parasites increased the morbidity, mortality and disability burden.

There is a lack of data regarding cryptosporidiosis and giardiasis outbreaks in Costa Rica; however, some studies have shown a high prevalence of infection in rural communities. Moore *et al.* (1966) reported that *Entamoeba histolytica*, *Giardia lamblia* and other intestinal protozoan parasites were responsible for 22% of diarrhea cases reported in both children and adults in the rural community of Canton of Barva located outside the capital city of San Jose. Another study, conducted in the rural mountain town of Puriscal, Costa Rica, found that 4.3% of feces sampled from infants and preschool children with diarrhea contained *Cryptosporidium* sp. oocysts (Mata *et al.* 1984). For this study, a clinical report was requested for the Monteverde region from the Costa Rican Department of Social Security, Northwest Central Pacific Health area. The report indicated that during the past three years, an annual average of 739 cases (14% of the population) of severe diarrhea was reported at the Monteverde clinic. Although recent epidemiological studies are not available, the presence of *Cryptosporidium* sp. and *Giardia* sp. on crops sold in open air markets in Costa Rica (Monge & Chinchilla 1996) suggests that these pathogens pose a public and occupational health risk, especially in rural and low-income areas where livestock waste management strategies are lacking. To improve the environmental sustainability of swine farms, the Costa Rican Ministry of Agriculture and Livestock recommends land application of the waste and the use of biodigesters for waste management and biogas production (Carvajal *et al.* 2007). However, land application of raw or treated livestock waste still poses a public health threat due to pathogen loading. It should also be noted that while pigs may not be the main reservoirs for *Giardia lamblia* and *Cryptosporidium parvum*, they are reservoirs that play a role in the chain of transmission (Heymann, 2015).

Although policies and regulations on management of livestock waste may be in place in developing countries, successful enforcement is often lacking due to lack of commitment by local and national authorities, lack of infrastructure for regular monitoring and lack of education on the negative environmental and public health consequences from mismanagement of livestock waste (Kinyua *et al.* 2016b). In countries where national and local commitment is evident, small-scale anaerobic digesters are promoted for

biogas (a mixture of methane and carbon dioxide) production from livestock waste. In addition to environmental and energy benefits, the use of small-scale anaerobic digestion systems to treat livestock waste in developing countries has several social, public health and agricultural benefits (Kinyua *et al.* 2016a, 2016b). However, pathogens may still be present in effluents from these systems (Chauret *et al.* 1999; Manser *et al.* 2015; Kinyua *et al.* 2016c). In developed countries, anaerobic digester temperature and hydraulic retention time can be monitored and controlled to promote greater inactivation of pathogens. In small-scale anaerobic digesters used in the developing world operating parameters can also be adjusted to promote greater pathogen inactivation; however, temperature control, vital to inactivating pathogens, may not be feasible as the digesters are typically operated at ambient temperatures. In a prior study in our laboratory, Kinyua *et al.* (2016c) investigated the effect of operating parameters and environmental conditions found in small-scale tubular digesters used to treat swine waste in rural Costa Rica on the fate and viability of *Cryptosporidium parvum* oocysts and *Giardia lamblia* cysts. Through laboratory experiments and mathematical modeling, we showed that the operating strategies and environmental conditions found in the field tubular digesters significantly decreased the concentration of (oo)cysts in the effluent compared to the raw swine waste. However, effluents from the digesters still contained viable *Giardia lamblia* cysts for 30 days and viable *Cryptosporidium parvum* oocysts for about 100 days after an outbreak. Therefore, determining the risk of infection from *Cryptosporidium parvum* and *Giardia lamblia,* associated with handling raw livestock waste and anaerobic digestion effluents, is critical in an effort to protect the health of communities in developing countries.

Quantitative microbial risk assessment (QMRA) is a useful tool that is used to estimate the risk of a health effect including infection, illness and or death to humans from exposure to pathogens. By carrying out a QMRA, management practices that reduce exposure can be put in place. A risk-based management strategy is more attractive than a treatment technology-based management strategy due to its flexibility, depending on the region, location, culture, socio-economic status and other community dependent variables (Razzolini *et al.* 2011).

Several studies have evaluated the concentration of *Cryptosporidium parvum* oocysts, and *Giardia lamblia* cysts and the risk of infection associated with exposure at various pathways to these pathogens in raw livestock waste, raw domestic wastewater and class B biosolids (Heitman *et al.* 2002; Hutchison *et al.* 2004; Brooks *et al.* 2012; Harder *et al.* 2014). Class B biosolids are domestic wastewater sludge that is treated through anaerobic digestion (35–60 °C), aerobic digestion, composting, air drying or lime stabilization (USEPA 2001). Class B biosolids produced from anaerobic digestion systems at municipal wastewater treatment facilities differ from effluents from small-scale anaerobic digestion systems treating livestock waste due to digestion treatment operating parameters and environmental conditions. Brooks *et al.* (2012) investigated the risk of infection from occupational exposure to soil contaminated with *Cryptosporidium parvum* from raw cattle waste and class B biosolids. The authors found that over the course of 30 days, the risk of infection was greater during exposure to soils fertilized with raw cattle waste (3×10^{-4}) compared to soil fertilized with class B biosolids (1×10^{-5}). This can be attributed to the lower concentration of *Cryptosporidium parvum* in the class B biosolids. Cooper (2012) investigated the risk of infection from *Giardia* sp., and *Cryptosporidium* sp. from consumption of crops irrigated with effluent from a tertiary treatment process in a municipal wastewater treatment plant. The annual risk of infection from *Giardia* sp., and *Cryptosporidium* sp. was 8.54×10^{-5} and 2.04×10^{-4}, respectively. These studies indicate that the treatment of domestic wastewater reduces the risk of infection at various exposure pathways.

However, there are no prior studies that have investigated how the treatment of livestock waste through small-scale anaerobic digestion systems in developing countries influences the risk of infection from *Giardia lamblia* and *Cryptosporidium parvum* when the effluent from the systems is used as a soil amendment. The overall goal of this study was to use QMRA to examine the occupational and public health risks associated with management of raw livestock waste and tubular digester effluents in two rural communities located in the northwest region of Costa Rica. The specific objectives that guided this research were: (1) to determine the exposure pathways based on relevant on-farm practices at the two communities; (2) to determine

the risk of infection from *Giardia lamblia* and *Cryptosporidium parvum* associated at each exposure pathway; and (3) to compare the risks between the two communities based on their livestock waste management strategies. Since small-scale anaerobic digestion systems are being promoted for bioenergy production in a number of developing countries, determining the risk of infection from *Giardia lamblia* and *Cryptosporidium parvum* associated with reuse of effluents is important in an effort to promote environmental sustainability and decrease public health concerns.

MATERIALS AND METHODS

Site description

This study was carried out in two rural communities located on the Pacific Slope of the Tilarán Mountain Range of Costa Rica. The first community, San Luis de Monteverde (N 10′ 16.973″ W 84′ 47.882″) is located in the province of Puntarenas, with an altitude range of up to 1,200 m above sea level and a population of approximately 500 people. The main economic activities in San Luis de Monteverde are small-scale farming and eco-tourism. Households typically have about 10 cows and four to 10 pigs. Out of about 100 households, eight installed tubular anaerobic digesters to promote energy production and reduce livestock waste pollution. Most farmers with tubular digesters have both cows and pigs. Three of the households with tubular digesters co-digest swine and cattle waste, four treat only swine waste and one treats only cattle waste. The biogas produced is sufficient to meet household daily energy demands for cooking for an average family of five people. Details of the tubular digester design, operation and performance are presented in Kinyua *et al.* (2016a).

The second community La Florida (N 10 ° 23′ 45.33″ W 84 ° 54′ 10.2492″), is located in the province of Guanacaste, with an altitude range of up to 900 m above sea level and has a population of approximately 150 people. La Florida is located close to the continental divide where the climate and presence of rich volcanic soils make it ideal for tropical dairy farming. The predominant dairy farming system produces high quality varieties of grass and cow breeds. Most dairy farms in La Florida are family owned and operated. The dairy farms have about 26–80 cows and most of the milk produced is sold to Costa Rica's largest dairy cooperative, Dos Pinos. Dos Pinos collects the milk from the dairy farmers and processes it to various dairy products that are sold in the domestic and international markets. To meet the milk quality demands set by the dairy cooperatives, the farmers spend about 51% of their annual budget importing cattle feed to sustain their productivity and 15% of their annual budget on electricity for milking and cooling purposes. There are about 25 dairy farms in La Florida. The cows are free range and waste from the cows is only collected during milking. This waste is disposed of by land application on the cattle pastureland. Only one farm treats their waste through composting. In an effort to reduce electrical costs and reduce the environmental burden of dairy farming, the farmers of La Florida are interested in installing tubular anaerobic digestion systems to treat their livestock waste.

QMRA model development

Pathogen identification

Low and high concentrations of *Cryptosporidium parvum* and *Giardia lamblia* (oo)cysts in the raw swine and dairy cattle waste and tubular digester effluent after a natural infection were taken from the literature and are shown in Table 1 (Nydam *et al.* 2001; Yui *et al.* 2014; Kinyua *et al.* 2016c). The (oo)cysts concentrations in raw swine waste were from a study that sampled waste from 334 pigs aged less than one month to more than a year old (Yui *et al.* 2014). Since pigs less than 6 months old have been shown to shed more *Cryptosporidium* sp. oocysts compared with older pigs (Yui *et al.* 2014) and most of the farmers in San Luis de Monteverde using tubular digesters have pigs less than 4 months old (Kinyua 2015), only the low and high concentrations of (oo)cysts for pigs less than 6 months old were used. The (oo)cysts concentrations in raw cattle waste were from a study that sampled waste from 478 calves for *Cryptosporidium parvum* and 1,016 calves for *Giardia lamblia* (Nydam *et al.* 2001). Like in young pigs, dairy calves were more susceptible to infection compared to older dairy cattle (Nydam *et al.* 2001). In La Florida, calves make up

Table 1 | Low and high concentrations of *Cryptosporidium parvum* and *Giardia lamblia* in raw livestock waste and tubular digester effluents

Parameter	Unit	*Cryptosporidium parvum*	*Giardia lamblia*
Raw swine waste[a]	(oo)cysts/g TS	1.55×10^4–1.49×10^5	8.14×10^3–1.99×10^5
Raw cattle waste[a]	(oo)cysts/g TS	9.09×10^4–3.89×10^{10}	7.43×10^3–3.80×10^7
Digester 1 effluent[b]	(oo)cysts/L	2.81×10^1–1.40×10^5	3.00×10^{-3}–9.26×10^4
Digester 2 effluent[b]	(oo)cysts/L	2.26×10^2–3.94×10^4	2.29×10^{-2}–2.39×10^4
Digester 3 effluent[b]	(oo)cysts/L	4.96×10^1–1.02×10^5	1.42×10^{-3}–6.27×10^4

[a]Concentrations estimated from reported values in the literature (Nydam *et al.* 2001; Yui *et al.* 2014).
[b]Concentrations estimated by applying modeling approach presented in Kinyua *et al.* (2016c).

about 20% of the total dairy cattle in the farms. In a prior study in our laboratory (Kinyua *et al.* 2016c) a tubular digester mathematical model was developed by combining laboratory inactivation studies of *Cryptosporidium parvum* and *Giardia lamblia* and field data on the operation of three tubular digesters treating swine waste in San Luis de Monteverde. The model was used to predict concentrations of (oo)cysts in tubular digester effluents after an outbreak. Briefly, it was assumed that pigs shed viable (oo)cysts in a sporadic pattern for 10–28 days following a second-order polynomial distribution. This concentration distribution was combined with the inactivation rates, (oo)cysts distribution coefficients, and individual tubular digester operating parameters to determine the effluent (oo)cysts concentration. Inactivation rates of 0.056 ± 0.013 and 0.16 ± 0.064 day^{-1} were estimated for *Cryptosporidium parvum* and *Giardia lamblia*, respectively (Kinyua *et al.* 2016c). The low and high concentrations predicted by that model were used for this study and are shown on Table 1. The lowest effluent concentration was taken as the value when a saturation point of 4 log removal was predicted by the tubular digester model in the three digesters for both parasites.

Dose response

Protozoan parasites have been found to follow an exponential dose-response model and the probability of infection during a one-time exposure to *Cryptosporidium parvum* or *Giardia lamblia* is expressed as:

$$P_i = 1 - \exp(-rd) \quad (1)$$

where P_i is the one-time pathogen exposure probability of infection (Haas *et al.* 1999) and d is the average dose of the exposure. Prior epidemiological studies on healthy human volunteers have estimated a dose response parameter of a single agent causing infection (r) from exposure to *Cryptosporidium parvum* and *Giardia lamblia* as 5.72×10^{-2} and 1.99×10^{-2}, respectively (Regli *et al.* 1991; Messner *et al.* 2001).

Exposure assessment

To gain insight into the farmers' livestock and tubular digester management practices, participatory observations at each of the farms was carried out for six weeks. Interviews were also conducted at each of the farms to evaluate knowledge, attitudes and practices related to handling, treatment and disposal of livestock waste. Interviews were conducted in Spanish at the field sites. The interview questions were structured to capture risk assessment constructs (type of livestock, livestock waste treatment and disposal methods, use of personal protective equipment, use of treated and untreated livestock waste to fertilize crops and livestock environment). Results from the interviews in San Luis de Monteverde and La Florida are presented in Table 2. Only eight households using tubular digesters in San Luis de Monteverde were interviewed and in La Florida, eight of the 25 dairy farmers in the region were interviewed. Mathematical equations for exposure assessment were derived from Brooks *et al.* (2012) and Mota *et al.* (2009) and values for model inputs are summarized in Table 3. The pathways that were considered in this study are fomite and soil contamination and crop contamination from runoff.

Table 2 | Livestock waste interview results

	Questions N	San Luis 8	La Florida 8
Type of livestock and poultry	Mainly pigs	12.5%	0.0%
	Pigs and cows	75.0%	0.0%
	Mainly cows	12.5%	100%
	Poultry	100%	37.5%
Livestock environment	Free range pigs	12.5%	0.0%
	Free range cows	100%	100%
	Penned pigs	87.5%	0.0%
	Penned cows	0.0%	0.0%
Treatment and disposal of raw livestock and poultry waste	Land application	37.5%	12.5%
	Tubular digester	100%	0.0%
	Other	0.0%	12.5%
Use of digester effluent to fertilize	Crops eaten raw	12.5%	not applicable
	Fruit trees and cooked crops	100%	not applicable
Use of raw waste to fertilize	Crops eaten raw	0.0%	12.5%
	Fruit trees and cooked crops	37.5%	12.5%
Personal protective equipment	When handling livestock waste	12.5%	0.0%

Table 3 | *Cryptosporidium* sp. parameters and probability distributions used in the uncertainty analysis

Parameter	Unit	Distribution	Mean	Standard deviation	References
A_{rm}	Mg TS/ha		6.57	n/a	Gale (2005)
E	Kg crops/ ha	Normal	2070	375	Brooks *et al.* (2012)
D_r			0.045	n/a	Brooks *et al.* (2012)
D_s			0.00175	n/a	Gale (2005)
F_r	%		10	n/a	Brooks *et al.* (2012)
F_{rm}	g/fomite		0.1	n/a	Gale (2005)
K_c	$\log_{10}$ (n)	Normal	4.0 (3 days)	0.4	Warnes & Keevil (2003)
K_f	$\log_{10}$ (n)	Normal	4.0 (1–4 days)	1.13	Anderson (1986)
K_s	$\log_{10}$ (n)	Normal	3.28 (63–84 days)	0.28	Hu *et al.* (1996); Olson *et al.* (1999); Hutchison *et al.* (2004)
K_w	$\log_{10}$ (n)	Normal	4.0 (70 days)	0.51	Olson *et al.* (1999)
T_c	%	Normal	0.043	0.032	Monge & Chinchilla (1996)
T_h	%	Normal	43	12	Rusin *et al.* (2002); Brooks *et al.* (2012)
T_m	%	Normal	36	3.33	Rusin *et al.* (2002); Brooks *et al.* (2012)
T_r	%	Normal	9.00	2.85	Trask *et al.* (2004)
T_w	%	Normal	10	1.0	Gale (2005)

Fomite contamination

Fomite contamination was considered when the farmers handle raw manure to dispose of it in the cattle pastureland or when preparing tubular digester influent. Fomite contamination was calculated assuming that a fraction of raw manure was transferred to a fomite, such as the handle of the shovel or bucket. During a single event exposure, with

no decay, the fomite pathogen concentration was calculated from:

$$C_f = C_{rm} \times F_{rm} \quad (2)$$

where C_f is the fomite pathogen concentration ((oo)cysts/fomite), C_{rm} is the pathogen concentration in the raw cattle and swine waste ((oo)cysts/g TS), and F_{rm} is the amount of raw waste transferred to a fomite (g/fomite). Fomite pathogen concentration, accounting for inactivation over time, was calculated as:

$$C_f = C_{rm} \times F_{rm} \times \left(\frac{1}{10_f^K}\right) \quad (3)$$

where K_f is (oo)cysts inactivation rates on a fomite ($\log_{10}$). Fomite log removal rates were linearly extrapolated from day 0 to 5.

Soil contamination

Only soil contamination from the use of tubular digester effluent was considered. After performing a participatory observation on livestock waste disposal methods and interviews with dairy farmers in the La Florida community, it was concluded that there was negligible exposure to farmers from soil contamination, as they disposed of the manure in the cattle pastureland and did not tend to this soil. To calculate the pathogen concentrations in the soil, inactivation rates of *Cryptosporidium parvum* and *Giardia lamblia* in the soil were calculated from:

$$C_s = C_{de} \times D_s \times \left(\frac{1}{10_s^K}\right) \times \left(1,000\frac{g}{kg}\right) \quad (4)$$

where C_s is the soil pathogen concentration ((oo)cysts/kg soil), C_{de} is the concentration of pathogens in the digester effluent ((oo)cysts/L), D_s is the soil dilution ratio (L of digester effluent/g of soil), and K_s is the (oo)cysts inactivation rates in the soil at 25 °C ($\log_{10}$). Soil log inactivation rates were linearly extrapolated from day 0 to 120.

Of the eight farmers using tubular digesters in San Luis de Monteverde, only one farmer used their digester effluent to fertilize crops eaten raw, such as tomatoes and lettuce. All other farmers with digesters used the effluent to fertilize corn, beans, fruit trees and root crops that were later cooked. Therefore, direct crop contamination from use of raw livestock waste and tubular digester effluent was not assessed in this study.

Crop contamination from runoff

Only indirect crop contamination from runoff for low hanging foods eaten raw (lettuce, cabbage and cilantro) was considered. To determine the concentration of pathogens on foods eaten raw, the concentration of pathogens in the raw cattle waste and tubular digester effluent deposited on a field was first estimated:

$$C_p = C_{rm} \times A_{rm} \times \left(10^6\frac{g}{Mg}\right)$$
or
$$C_p = C_{de} \times A_{de} \quad (5)$$

where C_p is the concentration of (oo)cysts per hectare ((oo)cysts/ha), A_{rm} is the application rate of raw cattle waste (Mg/ha), and A_{de} is the application rate of tubular digester effluent (L/ha). A_{de} was calculated as the tubular digester effluent flow rate divided by the area where the farmers apply their effluent. The concentration of (oo)cysts on crops contaminated by runoff was expressed as:

$$C_c = \frac{(C_p \times F_r \times T_r \times D_r \times (1/10^{Kw}) \times T_c \times (1/10^{Kc}) \times T_w)}{E} \quad (6)$$

where C_c is the concentration of (oo)cysts on crops contaminated by runoff ((oo)cysts/kg), F_r is the fraction of effluent or raw manure available for runoff, T_r is the percent of (oo)cysts that will be transferred from the effluent or raw manure to the runoff water, D_r is the (oo)cysts dilution ratio in the runoff water, K_w is the inactivation rate of (oo)cysts in water at 25 °C ($\log_{10}$), T_c is the percent attachment of (oo)cysts from the runoff water to crops, K_c is the inactivation rate of (oo)cysts on crops at 22 °C ($\log_{10}$), T_w is the percentage of soil particles that remain on the crops after washing, and E is the mass of crops

exposed to the contaminated runoff water (kg/ha). T_r was calculated assuming a 2.2–5.2% transfer per 63.5 mm of rainfall/hour for the Monteverde region (Trask *et al.* 2004). It was assumed that the (oo)cyst load would be diluted when the effluent or raw manure came into contact with the runoff water and this value was derived from Brooks *et al.* (2012) for lettuce. There is limited research on the percent attachment of *Cryptosporidium* sp. and *Giardia* sp. in runoff water to crops. However, several studies have recovered (oo)cysts from leafy crops irrigated with wastewater (Monge & Chinchilla 1996; Amorós *et al.* 2010; Rzeżutka *et al.* 2010). T_c values for this study were assumed to be the same as the recovery rates obtained by Monge & Chinchilla (1996) who investigated the presence of *Cryptosporidium* sp. and *Giardia* sp. in lettuce and cilantro leaves sold in markets in Costa Rica. Water and crop log inactivation rates were linearly extrapolated from day 0 to 14.

Risk characterization

Risk characterization combines the exposure assessment data with the dose response data to determine the risk of infection from the pathogens at the different exposure pathways. The concentration of (oo)cysts ingested (dose) is estimated as:

$$d = C_{ep} \times C_i \quad \text{or} \quad d = C_{ep} \times C_i \times T_h \times T_m \tag{7}$$

where d is the dose ((oo)cysts/dose), C_{ep} is the concentration of (oo)cysts at each exposure pathway, C_i is the amount of soil ingested during occupational activities (0.48 kg soil/day) or leafy crops consumed per day (0.292 kg leafy crops/day), T_h is the transfer of (oo)cysts from fomite to hand, and T_m is the transfer of (oo)cysts from hand to mouth.

Data analysis

A Monte Carlo sensitivity analysis was performed using Oracle Crystal Ball (Redwood City, CA) by calculating the rank-order correlation coefficient by running 10,000 trials with selected parameter values within the range previously reported in the literature (see Tables 3 and 4) to determine how input parameters affected the risk of infection (output). All the inputs parameters were considered uncertain with normal distribution. Rank-order correlation

Table 4 | *Giardia* sp. parameters and probability distributions used in the uncertainty analysis

Parameter	Unit	Distribution	Mean	Standard deviation	References
A_{rm}	Mg TS/ha		6.57	n/a	Gale (2005)
E	Kg crops/ ha	Normal	2,070	375	
D_r			0.045	n/a	Brooks *et al.* (2012)
D_s			0.00175	n/a	Gale (2005)
F_r	%		0.1	n/a	Brooks *et al.* (2012)
F_{rm}	g/fomite		0.1	n/a	Gale (2005)
K_c	$\log_{10}$ (n)	Normal	4.0 (3 days)	0.4	Warnes & Keevil (2003)
K_f	$\log_{10}$ (n)	Normal	4.0 (1–4 days)	1.13	Anderson (1986)
K_s	$\log_{10}$ (n)	Normal	2.78 (84 days)	0.39	Hu *et al.* (1996); Olson *et al.* (1999); Hutchison *et al.* (2004)
K_w	$\log_{10}$ (n)	Normal	4.0 (14 days)	0.27	Olson *et al.* (1999)
T_c	%	Normal	4.3	3.2	Monge & Chinchilla (1996)
T_h	%	Normal	43	12	Rusin *et al.* (2002); Brooks *et al.* (2012)
T_m	%	Normal	36	3.33	Rusin *et al.* (2002); Brooks *et al.* (2012)
T_r	%	Normal	9.00	2.85	Trask *et al.* (2004)
T_w	%	Normal	10	1.0	Gale (2005)

values lie between −1 and 1, and indicate the strength of the relationship between the input parameters and the output (risk of infection). The magnitude of correlation coefficient indicates the inputs impact on the output.

RESULTS AND DISCUSSION

Fomite contamination

The risk of infection from *Cryptosporidium parvum* and *Giardia lamblia* was estimated for occupational based exposure for an adult handling a wooden fomite for periods of 1 to 5 days. Inactivation of (oo)cysts on a wooden fomite was used because farmers in these two communities used shovels with wooden handles to prepare swine waste slurry for the tubular digesters and when disposing of raw cattle waste. The risk of infection from occupational exposure to a contaminated wooden fomite is shown in Table 5 for raw swine and cattle manure as well as tubular digester effluent. Even though the farmers rarely came into contact with the digester effluent, the risk of infection from a fomite contaminated with digester effluent was included for scenarios when handling the effluent may be necessary. For this exposure scenario 0.1 g of raw cattle or swine waste was assumed to be transferred to the wooden fomite, based on USEPA occupational transfer (Brooks *et al.* 2012). The low and high risk of infection indicated in the table are based on the low and high concentrations of *Cryptosporidium parvum* and *Giardia lamblia* in raw livestock waste and tubular digester effluents based on the literature. Occupational exposure to wooden fomites contaminated with raw cattle waste presented a greater risk compared to raw swine waste due to the higher concentration of (oo)cysts in the raw cattle waste.

A sensitivity analysis was performed and the rank-order correlation coefficient indicating the relationship between the input parameters and the risk of infections are shown later in Table 8. From this assessment, the uncertainty of the inactivation of (oo)cysts had the greatest impact on the uncertainty in risk of infection. The risk of infection from exposure to contaminated fomites may be affected mainly by the inactivation of (oo)cysts on wooden fomites. Inactivation of (oo)cysts on fomites is affected by surface

Table 5 | Risk of infection from *Giardia lamblia* and *Cryptosporidium parvum* during occupational exposure to contaminated wooden fomites. Day represents number of days after contamination of fomite

	Day	1 Low–High	2 Low–High	3 Low–High	4 Low–High	5 Low–High
Giardia lamblia	Raw swine waste	5.48×10^{-2}–7.4×10^{-1}	2.63×10^{-3}–6.22×10^{-2}	1.23×10^{-4}–3.00×10^{-3}	5.76×10^{-6}–1.41×10^{-4}	2.70×10^{-7}–6.57×10^{-6}
	Raw dairy cattle waste	5.01×10^{-2}–1.00	2.40×10^{-3}–1.00	1.12×10^{-4}–4.37×10^{-1}	5.26×10^{-6}–2.65×10^{-2}	2.46×10^{-7}–1.26×10^{-3}
	Digester 1	1.28×10^{-5}–2.33×10^{-1}	6.00×10^{-7}–1.23×10^{-2}	2.80×10^{-8}–5.79×10^{-4}	1.31×10^{-9}–2.71×10^{-5}	6.14×10^{-11}–1.27×10^{-6}
	Digester 2	8.39×10^{-7}–1.05×10^{-2}	3.92×10^{-8}–4.95×10^{-4}	1.83×10^{-9}–2.32×10^{-5}	8.58×10^{-11}–1.08×10^{-6}	4.01×10^{-12}–5.07×10^{-8}
	Digester 3	1.45×10^{-6}–2.68×10^{-2}	6.81×10^{-8}–1.27×10^{-3}	3.18×10^{-9}–5.93×10^{-5}	1.49×10^{-10}–2.78×10^{-6}	6.96×10^{-12}–1.30×10^{-7}
Cryptosporidium parvum	Raw swine waste	2.66×10^{-1}–9.48×10^{-1}	1.43×10^{-2}–1.29×10^{-1}	6.75×10^{-4}–6.44×10^{-3}	3.16×10^{-5}–3.02×10^{-4}	1.48×10^{-6}–1.41×10^{-5}
	Raw dairy cattle waste	8.36×10^{-1}–1.00	8.10×10^{-2}–1.00	3.94×10^{-3}–1.00	1.85×10^{-4}–1.00	8.65×10^{-6}–9.75×10^{-1}
	Digester 1	7.26×10^{-4}–9.73×10^{-1}	3.40×10^{-5}–1.55×10^{-1}	1.59×10^{-6}–7.86×10^{-3}	7.43×10^{-8}–3.69×10^{-4}	3.48×10^{-9}–1.73×10^{-5}
	Digester 2	1.00×10^{-3}–1.60×10^{-1}	4.68×10^{-5}–8.12×10^{-3}	2.19×10^{-6}–3.81×10^{-4}	1.02×10^{-7}–1.78×10^{-5}	4.79×10^{-9}–8.34×10^{-7}
	Digester 3	9.01×10^{-4}–1.45×10^{-1}	4.21×10^{-5}–7.31×10^{-3}	1.97×10^{-6}–3.43×10^{-4}	9.22×10^{-8}–1.60×10^{-5}	4.31×10^{-9}–7.51×10^{-7}

characteristics (porous or nonporous) and environmental conditions such as temperature, relative humidity and exposure to UV radiation (Bowman 2009). Anderson (1986) investigated the inactivation of *Cryptosporidium* sp. on a wooden surface at ambient temperature and reported a 4 log removal in 3 days. Other studies have investigated inactivation of *Cryptosporidium* sp. oocysts on dry metal surgical blades and dry glass surfaces and reported higher inactivation rates compared to wooden surfaces. This difference in inactivation could be due to cracks and crevices on wooden surfaces that may protect the oocysts from inactivation (Robertson *et al.* 1992; Barbee *et al.* 1999). It should be noted that the inactivation rate of *Giardia lamblia* on wooden fomites was assumed to be similar to the inactivation rate of *Cryptosporidium* sp. on a wooden surface due to lack of literature on *Giardia* sp. inactivation on fomites. This assumption may have overestimated the risk of infection from *Giardia lamblia*, as the inactivation of *Giardia lamblia* has been shown to be greater than *Cryptosporidium* sp. when the (oo)cysts are exposed to similar environmental conditions (Olson *et al.* 1999; Kinyua *et al.* 2016c). More research is required to investigate inactivation of *Giardia lamblia* on fomites to provide more accurate data for communities at risk of infection from these parasites. Additionally, risk management strategies, such as personal hand hygiene and placing shovels in the sun for parasite inactivation through UV radiation, can be encouraged to lower the risk of infection from occupational exposure to contaminated fomites.

Soil contamination

The risk of infection from *Cryptosporidium parvum* and *Giardia lamblia* was estimated for occupational based exposure for an adult tending to the soil after application of the tubular digester effluent for periods of 1 to 120 days. The risk of infection from (oo)cysts from using tubular digester effluents as a soil amendment are summarized in Table 6. Three conclusions were drawn from these results. First, the risk of infection from *Giardia lamblia* was significantly lower than the risk of infection from *Cryptosporidium parvum* for the same time periods ($p < 0.006$). Although the soil inactivation rates between the two parasites were not significantly different, tubular digester effluent (oo)cysts

Table 6 | Risk of infection from *Giardia lamblia* and *Cryptosporidium parvum* during occupational exposure to contaminated soil from tubular digester effluents. Day represents number of days after application of digester effluent on soil

Day		1 Low–High	7 Low–High	14 Low–High	21 Low–High	28 Low–High	60 Low–High	90 Low–High	120 Low–High
Giardia lamblia	Digester 1	4.65×10^{-5}–1.00	2.94×10^{-5}–1.00	1.73×10^{-5}–1.00	1.01×10^{-5}–1.00	5.94×10^{-6}–1.00	5.19×10^{-7}–1.00	5.27×10^{-8}–8.03×10^{-1}	5.36×10^{-9}–1.52×10^{-1}
	Digester 2	3.55×10^{-4}–1.00	2.25×10^{-4}–1.00	1.32×10^{-4}–1.00	7.73×10^{-5}–9.99×10^{-1}	4.54×10^{-5}–9.46×10^{-1}	3.96×10^{-6}–9.84×10^{-1}	4.03×10^{-7}–3.43×10^{-1}	4.09×10^{-8}–4.18×10^{-2}
	Digester 3	6.38×10^{-5}–1.00	4.43×10^{-5}–1.00	2.37×10^{-5}–1.00	1.39×10^{-5}–1.00	8.15×10^{-6}–1.00	7.11×10^{-7}–1.00	7.23×10^{-8}–6.67×10^{-1}	7.35×10^{-9}–1.06×10^{-1}
Crypto-sporidium parvum	Digester 1	6.98×10^{-1}–1.00	4.43×10^{-1}–1.00	2.23×10^{-1}–1.00	1.04×10^{-1}–1.00	4.62×10^{-2}–1.00	1.02×10^{-3}–9.94×10^{-1}	2.82×10^{-5}–1.31×10^{-1}	7.78×10^{-7}–3.85×10^{-3}
	Digester 2	1.00–1.00	9.91×10^{-1}–1.00	8.69×10^{-1}–1.00	5.85×10^{-1}–1.00	3.16×10^{-1}–1.00	8.21×10^{-3}–7.62×10^{-1}	2.27×10^{-4}–3.87×10^{-2}	6.25×10^{-6}–1.09×10^{-3}
	Digester 3	8.80×10^{-1}–1.00	6.44×10^{-1}–1.00	3.60×10^{-1}–1.00	1.76×10^{-1}–1.00	8.01×10^{-2}–1.00	1.81×10^{-3}–9.76×10^{-1}	4.98×10^{-5}–9.78×10^{-2}	1.37×10^{-6}–2.83×10^{-3}

concentrations differed significantly (see Table 1) due to differences in operating parameters between the three digesters and (oo)cysts inactivation rates during digestion (Kinyua *et al.* 2016c).

Second, the risk of infection during occupational exposure to contaminated soil was higher in this study than the reported risk of infection in other studies. Brooks *et al.* (2012) investigated the risk of infection to *Cryptosporidium parvum* from use of class B biosolids on soils. By day 7, the risk of infection was lower than 1×10^{-4}, the acceptable risk of infection according to WHO, while for this study the risk of infection was higher. The difference in the risk of infection is mainly due to the concentration of viable (oo)cysts in the tubular digester effluent compared to class B biosolids. Tubular digesters used in this study were operated at a temperature of approximately 21 °C, resulting in lower (oo)cysts inactivation rates (Kinyua *et al.* 2016c) compared with anaerobic digesters producing class B biosolids, which were operated under mesophilic (30–37 °C) and thermophilic (50–60 °C) temperatures. At 21 °C, log removal rates of 0.065 and 0.023 log removal/day were observed for *Giardia lamblia* and *Cryptosporidium parvum,* respectively (Kinyua *et al.* 2016c). At 36 °C, 0.15 log removal/day was observed for *Cryptosporidium parvum*, 3 log removal for *Giardia lamblia* and 1.0 log removal/day was observed under thermophilic temperatures (47–55 °C) (Kato *et al.* 2003; Gale 2005).

A Monte Carlo simulation was performed to determine how the uncertainty of the inputs parameters influenced the uncertainty of the risk of infection. These results are shown in Table 8 and indicate a negative correlation between the inactivation of (oo)cysts in soil and the risk of infection. Inactivation of (oo)cysts in the soil is affected by environmental conditions, such as temperature and moisture content. As the temperature of the soil increases, the inactivation of (oo)cysts would also increase leading to a lower risk of infection. The (oo)cysts inactivation rates in the soil were reported at 25 °C (Hu *et al.* 1996; Olson *et al.* 1999). Moisture content of the soil also influences the (oo)cysts inactivation rates in soil. As moisture content increases (oo)cysts inactivation rates decrease (Barwick *et al.* 2003). Moisture contents less than 1% result in desiccation/drying of pathogen membranes, which causes inactivation (Cotruvo 2004). Soil moisture content increases during rainfall events. The Monteverde region of Costa Rica, where this study was performed, has a mean annual temperature of 18.8 °C, with a mean annual precipitation of 2,519 mm. This region also houses the Monteverde Cloud Forest, where cloud cover leads to soil moisture contents of approximately 70% during the rainy season and 20–40% during the dry season (Nadkarni & Wheelwright 2000). Although soil moisture content was not incorporated in the soil contamination model, the high soil moisture content in Monteverde may decrease (oo)cysts inactivation rates in soil, thus increasing the risk of infection.

Crop contamination from runoff

The risk of infection from *Cryptosporidium parvum* and *Giardia lamblia* was estimated for consumption of crops contaminated with (oo)cysts in runoff water. The crop contamination from runoff model was based on leafy crops eaten raw due to dietary habits of households in La Florida and San Luis de Monteverde based on interviews. There is a lack of literature on the inactivation of *Giardia* sp. on crops, therefore, the crop inactivation rate of *Giardia lamblia* was assumed to be similar to that of *Cryptosporidium parvum*. Results from the crop contamination from runoff model are summarized in Table 7. From these results, two main conclusions were noted. First, several assumptions were made for the crop contamination from the runoff model. To determine how the uncertainty of these assumptions on the input parameters affected the uncertainty of the risk of infection, a Monte Carlo simulation was performed as described in the Data Analysis Section and the results are summarized in Table 8. The (oo)cysts inactivation rates on leafy crops (K_c) had the greatest negative correlation to the uncertainty of the risk of infection for both tubular digester effluents and raw cattle waste. *Cryptosporidium parvum* oocysts survival on crops has been shown to depend on the type of leaf, for example iceberg lettuce leaves versus parsley leaves, when the crops are stored at the same temperature (Warnes & Keevil 2003). Oocysts survive longer in crinkly textured and larger leafed crops, as the contours in the leaves provide the oocysts protection from desiccation. In smaller leaved crops, such as cilantro and parsley, the crop's shorter shelf life promotes desiccation as the crop dries up (Warnes & Keevil 2003).

Table 7 | Risk of infection from *Cryptosporidium parvum* and *Giardia lamblia* from consumption of leafy crops contaminated with runoff water. Day represents number of days after rainfall event

		1	3	7	11	14
Day		**Low–High**	**Low–High**	**Low–High**	**Low–High**	**Low–High**
Giardia lamblia	Raw cattle manure	5.77×10^{-3}–1.00	3.39×10^{-6}–1.72×10^{-2}	1.17×10^{-12}–5.98×10^{-9}	0.00–2.11×10^{-15}	0.00–0.00
	Digester 1	4.62×10^{-13}–1.43×10^{-5}	0.00–8.37×10^{-9}	0.00–2.89×10^{-15}	0.00–0.00	0.00–0.00
	Digester 2	3.36×10^{-13}–3.51×10^{-7}	0.00–2.06×10^{-10}	0.00–0.00	0.00–0.00	0.00–0.00
	Digester 3	1.98×10^{-13}–3.01×10^{-6}	0.00–1.77×10^{-9}	0.00–0.00	0.00–0.00	0.00–0.00
Cryptosporidium parvum	Raw cattle manure	2.91×10^{-1}–1.00	5.78×10^{-4}–1.00	1.64×10^{-9}–7.00×10^{-4}	4.66×10^{-15}–1.98×10^{-9}	0.00–1.37×10^{-13}
	Digester 1	2.11×10^{-8}–1.05×10^{-4}	3.54×10^{-11}–1.76×10^{-7}	0.00–4.97×10^{-13}	0.00–0.00	0.00–0.00
	Digester 2	1.61×10^{-8}–2.81×10^{-6}	2.71×10^{-11}–4.72×10^{-9}	0.00–1.33×10^{-14}	0.00–0.00	0.00–0.00
	Digester 3	1.16×10^{-8}–2.40×10^{-5}	1.95×10^{-11}–4.03×10^{-8}	0.00–1.14×10^{-13}	0.00–0.00	0.00–0.00

Second, it was noted that a one-time runoff event resulted in risks of infection greater than 10^{-4} from both parasites originating from raw cattle waste within the first day with significantly lower risk of infection when tubular digester effluent was land applied. These results indicate that if leafy crops are harvested one day after a runoff event in San Luis de Monteverde where farmers use tubular digester effluent as a soil amendment, the risk of infection from *Cryptosporidium parvum* and *Giardia lamblia* is significantly less compared to harvesting leafy crops in La Florida where cattle waste remains untreated. This indicates that the use of tubular digesters significantly reduces the risk of infection and illness from either giardiasis or cryptosporidiosis.

Risk simulation

Results from this risk assessment indicate that occupational exposure (fomite and soil contamination) resulted in higher risks than indirect exposure (crop contamination from runoff). This study is a good starting point to understand and predict the risk of infection of *Cryptosporidium parvum*, *Giardia lamblia* and other pathogens of concern, especially for communities in the developing world. In addition, the study indicates that tubular digesters can assist in reducing direct and indirect risks of infection, with these parasites. It should also be noted that farmers in both San Luis de Monteverde and La Florida land apply raw poultry waste on crops that are eaten raw; therefore, inactivation in tubular digesters and QMRA studies for other pathogens relevant to poultry waste should also be considered to reduce public health concerns from use of untreated poultry waste. The exposure pathways and risks estimated in this study did not account for variations in soil moisture content, wildlife contributions to (oo)cysts loads, continuous rainfall events or other environmental inactivation mechanisms such as UV radiation on soil. In addition, several assumptions were made including the inactivation of *Giardia* sp. cysts on wooden fomites (K_f) and lettuce (K_c) and the percent attachment of (oo)cysts from the runoff water to crops (T_r) due to lack of information in the published literature. This indicates that more research is needed in these areas on these two zoonotic parasites (*Cryptosporidium* sp. and *Giardia* sp.) to provide accurate predictions especially for communities in the developing world.

Table 8 | Rank-order correlation coefficients for *Giardia* sp. and *Cryptosporidium* sp. at three exposure pathways

***Giardia* sp.**

Parameter	Digester 1	Raw cattle manure	Raw swine manure
Fomite contamination			
Fomite inactivation ($K_{f)}$)	−0.72	−0.47	−0.73
Dose response parameter (r)	0.55	0.49	0.55
Transfer from fomite to hand (T_h)	0.23	0.17	0.23
Transfer from hand to mouth (T_m)	0.070	0.045	0.075
Soil contamination			
Dose response parameter (r)	0.83	–	–
Soil inactivation ($K_{s)}$)	−0.39	–	–
Crop contamination from runoff			
Crop inactivation (K_c)	−0.36	−0.49	–
Mass of contamination crops (E)	−0.12	−0.085	–
Water inactivation (K_w)	−0.040	−0.060	–
Soil particles remaining on crops after washing (T_w)	0.015	0.015	–
Dose response parameter (r)	0.21	0.17	–
Transfer of residual to runoff water (T_r)	0.43	0.39	–
Attachment from the runoff water (T_c)	0.63	0.58	–

***Cryptosporidium* sp.**

Parameter	Digester 1	Raw cattle manure	Raw swine manure
Fomite contamination			
Fomite inactivation ($K_{f)}$)	−0.79	−0.98	−0.79
Dose response parameter (r)	0.46	0.11	0.45
Transfer from fomite to hand (T_h)	0.26	0.11	0.27
Transfer from hand to mouth (T_m)	0.08	0.1	0.08
Soil contamination			
Dose response parameter (r)	0.62	–	–
Soil inactivation ($K_{s)}$)	−0.74	–	–
Crop contamination from runoff			
Crop inactivation (K_c)	−0.16	−0.050	–
Mass of contamination crops (E)	−0.085	−0.035	–
Water inactivation (K_w)	−0.040	−0.005	–
Soil particles remaining on crops after washing (T_w)	−0.005	0.010	–
Dose response parameter (r)	0.26	0.11	–
Transfer of residual to runoff water (T_r)	0.41	0.20	–
Attachment from the runoff water (T_c)	0.61	0.47	–

Other zoonotic pathogens such as Campylobacter jejuni and *E. coli* O157 may be of greater concern than the pathogens investigated in this study therefore, additional inactivation and QMRA studies should be carried out to understand the best management practices that can reduce the risks from these pathogens.

This study looked at tubular digesters as the main treatment method for raw livestock manure for rural

communities, however alternative treatment technologies such as composting and solar drying of the raw manure, should also be assessed to determine which treatment method results in the lowest risk at the various exposure pathways. Farmers with and without digesters still need to implement best management practices to control digester effluent and raw manure runoff. Some of these practices include: (1) a vegetation filter strip between their fields, grazing land and water bodies; (2) a water and sediment drainage basin that receives agricultural runoff and digester effluent; (3) constructed wetlands; and (4) duckweed and fish ponds (Miller *et al.* 2012).

CONCLUSIONS

This study investigated the risk of infection from *Cryptosporidium parvum* and *Giardia lamblia* from exposure to raw livestock waste and modeled (oo)cysts effluent concentrations at different exposure pathways. Since the (oo)cysts concentrations were higher in cow waste than swine waste, the risk of infection is greater when farmers handle wooden fomites that are contaminated with cow waste. The risk of infection from *Cryptosporidium parvum* during occupational exposure to contaminated soil from tubular digester effluents was higher than from exposure to *Giardia lamblia* due to higher inactivation of *Giardia lamblia* during anaerobic digestion. The risk of infection from *Cryptosporidium parvum* and *Giardia lamblia* from consumption of leafy crops contaminated with runoff water in San Luis de Monteverde where tubular digesters are in use was significantly lower than the risk of infection in La Florida where cattle waste was untreated.

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