

Prevalence and diversity of intestinal helminth eggs in pit latrine sludge of a tropical urban area

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ABSTRACT

The aim of the study was to investigate the prevalence and diversity of helminth eggs in pit latrine sludge in Yaounde, Cameroon. A total of 30 faecal sludge samples were collected in various latrines and analysed for physico-chemical parameters and helminth eggs' characterization was undertaken using standard protocols. Effects of physico-chemical parameters (pH, temperature, salinity, electrical conductivity, chemical oxygen demand, biochemical oxygen demand for 5 days, nitrogen ammonia, dry matter (DM), moisture content) on the parasite eggs were addressed. The total helminth egg concentration in the samples ranged from 8.5 eggs/g DM to a maximum of 264.5 eggs/g DM with a median of 81.1 eggs/g DM. Nematodes represented 67% of the total species followed by Trematodes and Cestodes. The helminth species with high prevalence in the sludge were *Ascaris lumbricoides* (41.4 eggs/g DM), *Ankylostoma duodenale* (31.5 eggs/g DM), *Fasciola hepatica* (34.9 eggs/g DM) and *Trichuris trichiura* (32.5 eggs/g DM). The physico-chemical parameters had no effect on the parasite concentration. Due to the high helminth egg concentrations in positive samples analysed, the need for proper health and environmental protection measures has to be stressed to prevent helminthic disease transmission due to untreated sludge discharge into the environment after pit latrine emptying or via direct agricultural use.

Key words | health consideration, parasite, pit latrine, tropical urban area, waste management

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INTRODUCTION

Adequate sanitation is an important foundation for health, economic development and well-being (Bartram & Cairncross 2010). In low- and middle-income countries, most urban dwellers (>70%) use mainly on-site sanitation systems such as pit latrines, public toilets and septic tanks for excreta and wastewater disposal (Tilley *et al.* 2014). In areas where access to sustainable sanitation, i.e., safe storage, collection, treatment and safe disposal/reuse of faeces and urine is insufficient or poor, parasites spread in the natural environment (Bartram & Cairncross 2010). Lack of sanitation leads to several diseases, and diseases associated with poor sanitation are particularly correlated with poverty and are responsible for about 10% of the

global burden of diseases. In fact, about half the urban population of Africa have diseases associated with poor sanitation, hygiene and water (Wolf *et al.* 2014). It has been stated that 1 g of fresh excreta from an infected person can contain around 10^6 viral pathogens, 10^6 – 10^8 bacterial pathogens, 10^4 protozoans cysts or oocysts and 10 – 10^4 helminth eggs. The geohelminth *Ascaris lumbricoides* is a global health concern because it infected nearly 800 million people in 2010, especially in tropical and subtropical regions (Pullan *et al.* 2014). The negative health outcomes of these infections are numerous and include morbidity due to nutritional impairment, negative impact on child growth, cognitive development and

worker productivity (Bethony *et al.* 2006). Helminth infections are acquired from an environment contaminated by worms' infective stages that developed from their fertilized eggs (Pullan *et al.* 2014). People infected with helminths pass eggs in their faeces, which will then mature in the environment before becoming infective again. Thus, the storage practice of human excreta is an important factor to reduce the risk of infections with helminths (Yen-Phi *et al.* 2010; Stenström *et al.* 2011).

The survival of parasites outside the host depends on environmental conditions. There are many abiotic and biotic factors, such as temperature, which affect resistant stages of parasite eggs (Manser *et al.* 2015). Penson *et al.* (2007) revealed that there are some factors that may contribute to the survival of *Ascaris* eggs in sewage sludge including temperature and pH. Another chemical factor known to affect the survival of helminth eggs is ammonia. It was shown that an increase of the pH may be favourable to the conversion of NH_4^+ to NH_3 , a chemical element which is known to inactivate many pathogenic organisms (Thangarajan *et al.* 2014). Inactivation of *Ascaris* eggs was found to be proportional to the concentration of NH_3 (Fidjeland *et al.* 2015). Also, lack of oxygen suppresses the overall metabolism of many nematodes (Etewa *et al.* 2014). The biological factors that have been shown to affect parasite eggs include the presence of fungi and various invertebrates. Results from de Carvalho *et al.* (2014) indicated that ovicidal fungi were capable of attacking and destroying *Ascaris lumbricoides* eggs. Additionally, desiccation (solar drying), which is a parameter depending on the water content, may influence the survival of helminth eggs. Indeed, the observations made by Seck *et al.* (2015), working on the effects of solar drying on helminth eggs' removal in unplanted drying beds treating faecal sludge (FS) under tropical conditions revealed high effects of solar drying on the die-off of helminth eggs.

The presence of helminth eggs in FS limits its options for reuse (Nelson *et al.* 2004; Maya *et al.* 2012). Despite the potential health risks, human excreta is a valuable resource as a fertilizer for agricultural production (Appiah-Effah *et al.* 2015). Excreta contains nutrients such as phosphorus, nitrogen and potassium, which are essential to plant growth. Thus, application of excreta in agriculture can help communities to increase agricultural

productivity through the recycling of nutrients while saving on cost for chemical fertilizers, resulting in economic benefits (Buit & Jansen 2016). The use of human excreta is a common practice that has long been part of the agricultural tradition in Vietnam (Yen-Phi *et al.* 2010). Various biological processes have been studied to determine the effectiveness of parasites' inactivation in domestic wastewater/FS during treatment processes (Jimenez *et al.* 2000). However, there is limited knowledge about the survival, the prevalence and diversity of parasites in the storage devices. Knowledge of the prevalence and diversity of parasites in pit latrine sludge could contribute to the choice of appropriate technologies for the inactivation of parasites during storage before treatment. In this study, the diversity and prevalence of helminth eggs in pit latrine sludge were assessed as well as the relation between the physico-chemical parameters and the helminth egg content.

MATERIALS AND METHODS

Study area context

The study was carried out in Yaounde (Cameroon), an urban area of about 256 km², limited by latitudes 03°45' and 04° N and longitudes 11°20' and 11°40' E (Figure 1). The town is located at about 700–800 m above sea level and had an estimated population of 2.5 million inhabitants in 2011 (BUCREP 2012). Yaounde has an equatorial climate with four seasons: two dry seasons (December–February, July–August) and two rainy seasons (March–June, September–November). The average annual rainfall is 1,600 mm with an average temperature of 23 °C. Facing overpopulation, like many other towns in developing countries, a large part of the capital consists of informal settlements with very basic water supply, sanitation and waste disposal infrastructures (Parrot *et al.* 2009). On-site sanitation systems for excreta collection are widespread, with the predominance of pit latrines (>59%). The city does not have any FS treatment plants and it was estimated that about 700–1,300 m³ of FS is discharged weekly into the environment of peri-urban areas (Figure 1) (Berteigne 2012).

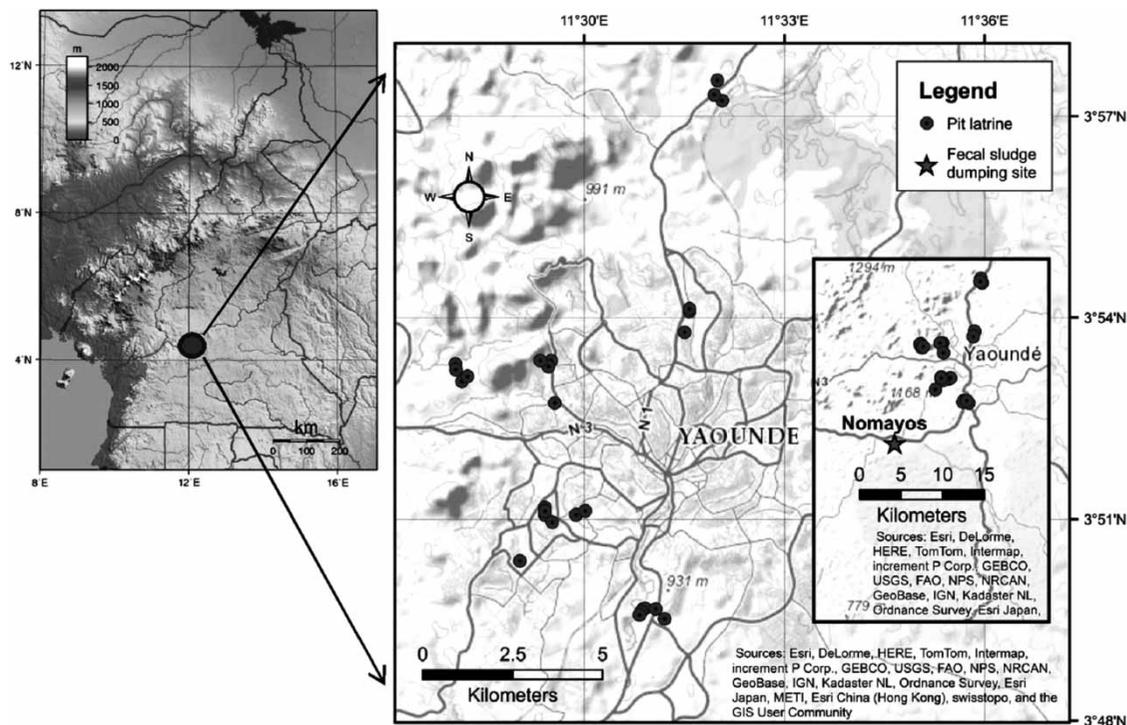


Figure 1 | Spatial distribution of the pit latrines sampled in the study area as well as the illegal FS dumping site (Nomayos).

Assessment method

Prior to the collection of raw sludge samples in pits, a survey of 602 households was conducted in informal settlements and planned settlements of the town to assess the household on-site sanitation facilities' typology as well as their excreta management. Only 53 of the 602 investigated households issued a favourable response for the sampling of their pits, among which 30 were traditional latrines, 13 were septic tanks, 6 were ventilated improved pit latrines and 4 piped equipped latrines (latrines having a pipe for the release of excreta during flooding events). The depth of the pits for latrines investigated and the number of users varied widely from less than 2 m to more than 8 m and from less than 4 persons to more than 10 persons, respectively, for the depth and the number of users.

Only data for sludge from traditional pit latrines are presented in this paper. The sampling material was composed of a metal bar of 1.5 m in length and 3 cm diameter (Figure 2(a)). The metal bar was linked to a metallic box (10 cm in diameter and 20 cm in height), welded at its lower end for collecting the sludge, and the upper end was

connected to a 15 m graduated rope. The use of the rope allowed the sampling team to sample deep latrines found in the study area. The mass of the metal bar allowed the easy entrance of sampling material into the pit sludge substrate.

The sampling method took into account a number of considerations including a non-destructive operation: removal of sludge through the hole of the slabs in the latrine and easy cleaning after the sampling operations. Sampling was performed in the first 2 m of the sludge layer after vigorous stirring with the device. Additionally, the depth of the pits (the height between the surface of the sludge layer and the slab of the latrine) was measured as well the information about the size of the pits, the number of persons using the latrines and the presence of other material in the pits. Observations made in the field during sampling showed that the pits investigated were still being loaded with fresh faeces and all of them were simultaneously used for bathing. Additionally, solid wastes were found in the investigated pits and consisted of menstrual hygiene products, plastic materials, papers, clothes, baby diapers, broken glasses, and other waste materials (Figure 2(b)).



Figure 2 | Solid wastes found in the pits of the latrines investigated during FS sampling: (a) clothes and (b) a mixture of wastes in a shallow pit.

Analysis of samples

Physico-chemical parameters such as pH, electrical conductivity (EC), salinity, chemical oxygen demand (COD), biochemical oxygen demand for 5 days (BOD_5), nitrogen ammonia (NH_4^+), moisture content and dry matter (DM) are known to affect helminth eggs' survival in the environment. The parameters were chosen to assess their potential effects on the prevalence and diversity of helminth eggs in this study. pH was chosen because of its indirect role in the inactivation of helminth eggs and water chemistry processes. EC and salinity are known to affect the surrounding environment by their osmotic effects. The nitrogen ammonia (NH_4^+) and the moisture content (affected by the desiccation rate) are known to inactivate helminth eggs in the environment. DM is known to affect the concentration of helminth eggs in sludge, as they constitute a part of the total solids and behave as particles in solution.

Analyses were made following standard protocols for water and wastewater analysis (APHA/AWWA/WEF 2005). Parasitic characterization of samples was performed by the determination of their concentrations and diversities of helminth eggs. This determination of helminth eggs was performed following the protocol described by Schwartzbrod (2003). The method was based on the separation and concentration of sludge solid fractions in an interphase of ethyl acetate solution and flotation with a solution of zinc sulphate with a relative density of 1.18. Samples

were observed and identification of the different species of helminth eggs was made using the key for identification of intestinal parasites of the World Health Organization (WHO 2006). The number of eggs was counted in a McMaster chamber and the prevalence was calculated according to the following formula: $N = YM/CV$, with N = number of eggs per litre of sample; Y = mean number of helminth eggs counted in the different chambers observed (because three chamber McMaster cells were used in this study); M = volume of final product (mL); C = volume of the McMaster cell (0.3 mL); V = volume of sample (L).

Statistical analysis

Data analyses were performed using the software SPSS 22. Pathogens' detection frequencies as well as the distribution of their diversity in samples were examined. Descriptive statistics of the samples were undertaken to express the parameters' dispersion. Due to the non-normal distribution in the prevalence and diversity of parasites in this study, Spearman rank correlation test was computed to assess the relationship between helminth egg species and the total helminth eggs in samples as well as the description of the relationship between the parasitic and physico-chemical parameters of the sludge. Levene's test for homogeneity of variance was used to assess the parameters' dispersion. The level of significance was set to $p < 0.05$ for the analysis of variance.

RESULTS AND DISCUSSION

Physico-chemical characteristics of sludge

The storage duration of sludge in the latrine pits was found to be highly variable among the latrines investigated. It varied between a period of less than 2 years to more than 20 years. The mean value of the pit operation period before emptying, recorded in this study, was 8.4 years with standard deviation of 6.01.

The physico-chemical characteristics of the sludge samples of the 30 pit latrines are shown in Table 1. Levene's test for homogeneity of variance revealed a significant variation between the 30 samples analysed, considering the physico-chemical parameters tested. Regarding the pH, the minimum value measured was 5.9, attaining a maximum of 8.9 with a median of 7.2, showing that the pit latrine sludge pH studied is slightly basic. Samples also exhibited a high solids content with a median value of 44.8% of DM. Medians for EC and salinity were, respectively, 4.8 mS/cm and 2.4‰.

The high concentrations illustrate that the samples were strongly concentrated by inorganic dissolved solids and cations. Very high variation could be seen in the distribution of organic contents in the samples. Values recorded for COD varied between 0.4 g O₂/L and 124.2 g O₂/L, with a median value of 34.0 g O₂/L. High variation of BOD₅ concentration was also noted with a median value of 3.2 g

O₂/L. As for other physico-chemical parameters, the distribution of NH₄⁺ showed a high heterogeneity, and varied between 0.01 and 2.5 g/L, with a median value of 0.3 g/L. The median value of moisture content found in this study was about 55.2%, and this fell within the range reported in the literature (50–60% of the total weight; Bakare *et al.* 2012).

However, water content values of more than 80% were recorded by Radford & Fenner (2013) working on the fluidization of synthetic pit latrine sludge. The variation in the concentration of FS parameters has been mentioned by several authors (e.g., Strauss *et al.* 1997; Bassan *et al.* 2013). The findings of Bakare *et al.* (2012) revealed that the variation of sludge from on-site sanitation systems can be attributed to household habits and local environmental conditions. This includes differences in storage duration, toilet usage, inflow and water infiltration into the latrine pits.

The higher values of organic matter (BOD₅ and COD) obtained in samples could be explained by the variation of biodegradation process taking place in the latrine pits. Chen *et al.* (2014) mentioned the influence of anaerobic degradation processes taking place in on-site sanitation systems on FS characteristics. The rate and intensity of these anaerobic processes vary according to the system design, the retention periods and the presence of inhibiting substances. The findings of this study correspond to those obtained by Kengne *et al.* (2008), who characterized 44 sludge samples provided by vacuum trucks in an FS treatment plant in Yaounde (Cameroon). The authors pointed

Table 1 | Descriptive statistics of the physico-chemical parameters of sludge samples (n = 30)

| Sludge parameters | pH | EC (mS/cm) | Salinity (‰) | COD (g O ₂ /L) | BOD ₅ (g O ₂ /L) | NH ₄ ⁺ (g/L) | TKN (g/L) | DM (%) | Moisture content (%) |
|-----------------------------|---------|------------|--------------|---------------------------|--|------------------------------------|-----------|--------|----------------------|
| Minimum | 5.9 | 0.2 | 0.1 | 0.4 | 0.1 | 0.01 | 0.04 | 32.7 | 44.4 |
| Maximum | 8.9 | 17.2 | 10.1 | 124.2 | 18.5 | 2.5 | 4.9 | 55.5 | 67.3 |
| Median | 7.2 | 4.8 | 2.4 | 34.0 | 3.2 | 0.3 | 1.7 | 44.8 | 55.2 |
| Std deviation | 0.8 | 3.8 | 2.5 | 34.1 | 4.2 | 0.6 | 1.6 | 6.3 | 6.3 |
| Percentile 25 | 6.74 | 3.3 | 0.7 | 14.2 | 2.1 | 0.2 | 0.9 | 38.5 | 52.5 |
| Percentile 75 | 7.9 | 8.9 | 4.7 | 58.2 | 7.7 | 1.2 | 3.5 | 47.7 | 61.9 |
| Kengne <i>et al.</i> (2008) | 6.5–9.3 | | | 30.5 | | 0.4 | 0.9 | 2.9 | |
| Heinss <i>et al.</i> (1998) | | | | 20–50 | | 2–5 | | ≥3.5 | |
| Bassan <i>et al.</i> (2013) | | | | 12.4 | 2.1 | | | | |

out that there is a significant difference between samples according to the distribution of the parameters' DM, pH, COD and NH_4^+ . Differences observed between the concentration of DM recorded in this study and the results of Kengne et al. (2008) could be explained by the fact that FS collected directly from on-site sanitation systems (this study) did not have the same quality as FS being discharged at treatment plants, because water is frequently added into the pit to dilute FS during mechanical emptying.

Effect of physico-chemical characteristics of FS on the prevalence of helminth eggs

Our results demonstrated no significant effects of the physico-chemical parameters on the prevalence of helminth eggs (Spearman rank correlation; 0.05 level).

Hygienic quality of sludge

Helminth eggs in sludge may pose significant health risks to exposed populations through direct or indirect contact. Helminth eggs were detected in 100% of pit latrine sludge tested, corroborating the study of Yen-Phi et al. (2010) in Vietnam. Berteigne (2012) estimated that about 900 to

1,350 m^3 of untreated FS is discharged weekly into the peri-urban area of Yaounde (Cameroon). Thus, treatment is necessary to minimize the risk of helminth infection by excreta. This study revealed heterogeneity in the distribution of helminth species and the total helminth eggs (Table 2). The detection frequencies among individual helminth species varied significantly. *Ascaris lumbricoides* was detected in over 70% of all samples, which is in line with the results of previous studies (Koné et al. 2007; Yen-Phi et al. 2010), indicating a high prevalence of Ascariasis in the population. Indeed, observations by Nkengazong et al. (2010), assessing the prevalence of geohelminths in 420 Cameroonian pupils, revealed a high prevalence of *Ascaris* eggs. The predominance of *Ascaris* eggs in the sludge samples could also be explained by high egg production (200,000 eggs/day) and durable eggs (Feachem et al. 1983). The parasite *Ankylostoma duodenale* had the lowest detection frequencies (13.79%) (see Table 2). Nematodes represented 67% of the total species, followed by Trematodes and Cestodes. The helminth species prevalence (median values) found in samples were *A. lumbricoides* (41.4 eggs/g DM), *A. duodenale* (31.5 eggs/g DM), *Fasciola hepatica* (34.9 eggs/g DM), *Trichuris trichiura* (32.5 eggs/g DM), *Strongyloides stercoralis* (24.8 eggs/g DM), *Taenia* sp.

Table 2 | Concentration of helminth eggs found in FS

| Helminth eggs | n | Median | | Minimum | | Maximum | | Std deviation | | Percentile | | | | |
|----------------------------------|----|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| | | Eggs/L ^a | Eggs/g ^b | Eggs/L ^a | 25 | | 75 | |
| | | | | | | | | | | | Eggs/L ^a | Eggs/g ^b | Eggs/L ^a | Eggs/g ^b |
| <i>Ascaris lumbricoides</i> | 21 | 16,667 | 41.4 | 667 | 1.9 | 36,667 | 90.3 | 11,039 | 26.8 | 6,667 | 13.9 | 21,833 | 60.9 | |
| <i>Ankylostoma duodenale</i> | 4 | 16,611 | 31.5 | 4,222 | 9.4 | 32,000 | 81.0 | 12,262 | 31.6 | 9,306 | 17.4 | 29,556 | 71.4 | |
| <i>Enterobius vermicularis</i> | 11 | 8,667 | 22.7 | 3,333 | 7.1 | 20,000 | 61.1 | 7,004 | 19.7 | 4,167 | 8.9 | 19,778 | 38.7 | |
| <i>Fasciola hepatica</i> | 7 | 16,222 | 34.9 | 1,111 | 2.8 | 30,000 | 66.8 | 9,098 | 20.3 | 10,778 | 20.1 | 18,889 | 37.5 | |
| <i>Hymenolepis nana</i> | 7 | 5,556 | 13.4 | 3,333 | 6.6 | 16,667 | 48.8 | 4,572 | 14.3 | 4,389 | 10.0 | 7,556 | 18.6 | |
| <i>Schistosoma mansoni</i> | 8 | 11,389 | 22.8 | 4,444 | 8.5 | 32,222 | 79.7 | 9,867 | 25.5 | 5,805 | 12.2 | 21,833 | 51.7 | |
| <i>Strongyloides stercoralis</i> | 13 | 10,889 | 24.8 | 4,333 | 8.5 | 40,889 | 107.2 | 11,838 | 27.5 | 7,333 | 18.9 | 18,778 | 37.0 | |
| <i>Taenia</i> sp. | 11 | 10,000 | 24.7 | 667 | 1.7 | 26,667 | 64.2 | 8,815 | 19.0 | 8,333 | 18.2 | 22,444 | 45.4 | |
| <i>Trichuris trichiura</i> | 6 | 13,444 | 32.5 | 4,445 | 9.3 | 22,889 | 67.0 | 8,432 | 22.7 | 5,945 | 17.5 | 20,722 | 56.0 | |
| Total helminth eggs | 30 | 33,222 | 81.1 | 37,778 | 8.5 | 100,889 | 264.5 | 30,522 | 74.5 | 15,222 | 38.9 | 70,889 | 155.2 | |

^aHelminth eggs per litre of sludge.

^bHelminth eggs per gram of DM.

(24.7 eggs/g DM), *Enterobius vermicularis* (22.7 eggs/g DM) and *Hymenolepis nana* (13.4 eggs/g DM). The diversity of helminth species found in the samples is in accordance with Jimenez *et al.* (2000), who characterized wastewater sludge in Mexico and Yen-Phi *et al.* (2010), who characterized FS in Vietnam. The high variability in terms of number and diversity of helminth eggs could be attributed to the health status of pit latrine users, the epidemiological conditions of the populations in the study area as well as the storage condition in the pit of the latrines. By assessing pathogens in septage in Vietnam, Yen-Phi *et al.* (2010) recorded a correlation between helminth ova concentration and retention time of FS. In Vietnam, in order to reduce human health risks associated with excreta use in agriculture, the Vietnamese Ministry of Health has stipulated the time for human excreta storage in latrines to be at least six months before application as fertilizer (Jensen *et al.* 2008). Strauss *et al.* (1997) reported the effect of storage duration on FS stabilization. The effects of retention time could not be applied to explain the prevalence and diversity of helminth eggs recorded in our study as pits were sampled while in use.

The most prevalent helminth eggs found among the nine species identified is *A. lumbricoides*, which is in accordance with data reported in the literature stating that Ascariasis is globally one of the most frequent causes of helminthic infections (Pecson *et al.* 2007). Furthermore, *Ascaris* eggs are also characterized by their high resistance to environmental conditions, such as pH, temperature, desiccation, etc. (Ingallinella *et al.* 2002; Cofie *et al.* 2006; Koné *et al.* 2007). As the observed parasite egg concentrations in samples were higher than the limit recommended in the WHO (2006) guidelines for safe use of excreta in agriculture (<1 egg/g of DM), the need for proper health and environmental protection measures has to be stressed, in order to avoid the transmission of helminthic diseases through untreated sludge discharge into the environment or direct agricultural application. Mara & Sleight (2010) pointed out that the persistence of helminth eggs in the environment is the most important risk factor for disease transmission. To prevent disease transmission, it is necessary to apply particular interventions for safe disposal and storage of excreta. The number of species found varied from 1 to 6 per sample with more than 70% of the sample containing more than one

species. The difference in the number of helminth species found could be explained by the different transmission routes of helminth eggs and the latrine user's specific risks factors. Traub *et al.* (2004) demonstrated that the transmission of helminth eggs is influenced by several factors like micro-climate, sanitation, hygiene and environmental contamination with human excreta.

Our findings could contribute to the knowledge of helminthiasis epidemiology in the studied area. Furthermore, the presence of many helminth species in FS could result in co-infections (poly-parasitism) of farmers using faecal matter for crop fertilization. In the tropics, it is common for a single individual to be infected with several parasite species at the same time (Jensen *et al.* 2008). Assessment of the relationship between parasite species and the total helminth eggs using Spearman rank correlation was done. Significant relationships at 0.05 level were observed between *S. stercoralis* and *A. lumbricoides* ($R^2 = 0.620$), *A. lumbricoides* and the total helminth eggs ($R^2 = 0.694$), *S. stercoralis* and the total helminth eggs ($R^2 = 0.863$), *T. trichiuria* and the total helminth eggs ($R^2 = 0.829$). The said correlation could be explained by the fact that the helminth species involved in the correlation presented a similar distribution/pattern according the detection frequency in samples.

CONCLUSION

The prevalence and diversity of helminth eggs detected in pit latrine sludge collected from various locations in Yaounde indicate an infectious risk if the sludge is not properly managed. The sludge showed high variations of physico-chemical parameters and helminth egg concentrations. Nevertheless, physico-chemical parameters were not found to be associated with the prevalence of helminth eggs. All samples were characterized by a very high load of helminth eggs, thereby constituting a very high health risk for the population. Based on these findings, there is a need for further assessment on the public health implications of helminth infections as well as the sludge treatment process for parasite die-off or inactivation. In order to minimize the risks of infections along the FS management chain, investigations of helminthic organisms in sludge should receive

more attention for health planning and environmental protection measures.

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