Implications of soils around domestic water points in the spread of intestinal parasites in the city of Yaounde (Cameroon)

Ajeagah Gideon Aghaindum, Asi Quiggle Atud and Okoa Amougou Thérèse Nadège

ABSTRACT

To determine the role of soils in dissemination of enteric protozoan cysts and intestinal eggs and/or larvae of helminths, a study was carried out on muddy soils around springs and wells of six districts in Yaounde, Cameroon from February to July 2015. Protozoan cysts and helminth eggs and larvae were observed microscopically after concentration by standard scientific methods. Flagellated enteric protozoa cysts were detected at an average density of 245 ± 145 cysts/L for *Giardia intestinalis*, 308 ±190 cysts/L for *Chilomastix mesnili*, 105 ± 106 cysts/L for *Enteromonas hominis* and 96 ± 118 cysts/L for *Retortamonas intestinalis*. Cyst densities were higher during the short rainy season (277 ± 119 cysts/L) than in the short dry season (147 ± 60 cysts/L). The helminths identified were *Ascaris* sp., *Enterobius* sp., *Necator americanus* and/or *Ancylostoma duodenale*, *Strongyloides* sp., *Taenia* sp., *Hymenolepis* sp., *Diphyllobothrium* sp. and *Fasciola* sp. Size varied between 40 μm and 200 μm for eggs and between 100 μm and 600 μm for the larvae assessed. Densities of environmental forms of the helminths were also higher during the short rainy season (176 ± 77 agents/L) than during the short dry season (117 ± 49 agents/L). These results show that muddy soils could contribute to the contamination of wells and springs and should be considered in epidemiological studies of intestinal parasites.

Key words | contamination, helminth eggs/larvae, protozoa cysts, springs, soils, wells, yaounde

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INTRODUCTION

Access to drinking water and sanitation is a major problem in the cities of developing countries (Lenton 2007). The lack of access to drinking water and sanitation is at the root of environmental degradation and the emergence of water-borne diseases (Hutton *et al.* 2007). The agents responsible for biological contamination include viruses, bacteria, fungi, protozoa and helminths. Most of this pollution is of faecal origin, due to the spread of human and animal wastes. These water-borne pathogens cause nearly 99% of diarrheal diseases, according to the World Health Organization (WHO 2011). The WHO (2004) reported that worldwide infectious and parasitic diseases account for 9.5 million deaths a year (16.2% of all deaths recorded). Many households carry out poor water management, so the water is unable to find appropriate drainage channels. This water originates mostly from springs and wells that are close to homes. Their exploitation for household activities leads to the release of wastewater or stagnant water which mixes with top soil and organic matter to form mud that is spread around the vicinity. In a study on environmental parasitology Ajeagah *et al.* (2016) showed contamination of springs and wells by the resistant forms of entero-parasites. To better understand the potential sources of groundwater contamination, it is important to collect, evaluate and

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analyse soils around wells and springs. The objective of this research is to assess the role played by soils around wells and springs in the spread of intestinal parasitic forms.

MATERIALS AND METHODS

Study site

Our study site in Yaounde, the political capital of Cameroon, lies between latitude 3°51′ and 3°52′ North and between longitude 11°31′ and 11°33′ East (Suchel 1972). It is located at nearly 750 m altitude, characterized by a seasonal climate known as the 'Yaoundéen climate' (Suchel 1972) comprising a long dry season (LDS) which extends from mid-November to mid-March, a short rainy season (SRS) which runs from mid-March to the end of May, a short dry season (SDS) from June to August and a long rainy season (LRS) which extends from September to mid-November.

Sampling points

The study proceeded in two phases. The first phase was the prospection from November 2014 to January 2015. Multiple field trips made it possible to choose the sampling points according to their proximity to springs and wells and to human activity. Samples were chosen, from six muddy areas in the surroundings of springs denoted as MoS, NkS, MeS, MlS, EtS and ObS and six points in the surroundings of wells denoted as MoW, NkW, MeW, MlW, EtW and ObW in the districts, respectively, of Mokolo, Nkomkana, Melen I, Melen VII, Etoug-Ébé and Obili as presented in Figure 1. The second phase was the sampling which extended over 6 months from February to July 2015, and samples were collected monthly.

Description of some intake points

Overall, the samples resulting from wastewater, stagnant water and domestic effluents (muds) in the surroundings of springs

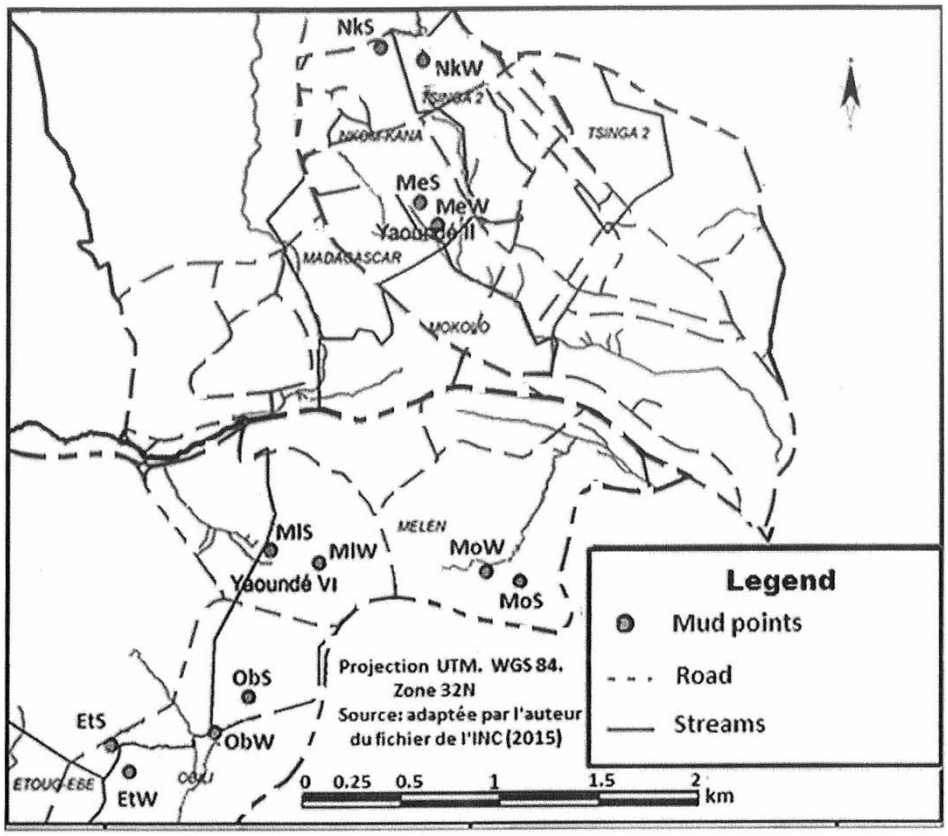

Figure 1 | Map of the locations of sampling points.

and wells near dwelling houses (less than 10 m distance from the springs and wells as presented in Table 1). Water from the springs and wells around the collecting points is usually used for drinking, bathing, teeth cleaning, cooking food, watering crops around the living areas and other domestic activities. Ajeagah *et al.* (2016) demonstrated the contamination of this groundwater by parasites around the study areas.

Sampling of muds for parasitic elements

Sampling for the identification of the dissemination forms of protozoa and helminths was carried out in muds at places characterized by an accumulation of organic matter. The mud samples collected were immediately introduced into sterile polyethylene bottles of 1,000 mL then transported to the Laboratory of Hydrobiology and Environment in a refrigerator. At the laboratory, the samples were stored for 24–48 hours before decanting. The supernatant was discarded and the pellet was measured and kept for parasitological analysis by various methods.

Sedimentation method

After homogenization of the pellet, 5 mL of sample was collected and placed in a test tube. To this, 1 mL of 10% formaldehyde (fixative), 5 mL of distilled water and two drops of Lugol's iodine were successively added. The mixture was centrifuged at 500 rpm for 5 minutes using a laboratory centrifuge (MEDIFRIGER). Subsequently, a drop of sample was removed, placed on a microscope slide and covered with a cover slide for identification and enumeration.

Zinc sulphate flotation or faust technique

The Faust technique was described by Faust *et al.* (1938). This method enables the suspension of cysts in water. After homogenization of the pellet, 5 mL of the sample was collected and placed in a test tube. To this, 1 mL of 10% formaldehyde and 3 mL of 33% zinc sulphate (specific gravity 1.18) were successively added. The mixture was centrifuged at 500 rpm for 5 minutes. A drop of the supernatant, in which the parasites can be found, was taken and spread on a slide which was then covered with a cover slip. Sedimentation and flotation methods were used for the observation of protozoa cysts.

Kato-Katz technique

For the Kato-Katz technique, a portion of the pellet produced by centrifugation at 500 rpm for 5 minutes was taken and deposited on the centre of a slide. The latter

Table 1 | Geographic coordinates of sampling points and distance to toilets

Sampling point	Geographic coordinates	Altitudes (m)	DM-W/S[a] (m).	DM-T[b] (m)
MoS	03°52′48.5″N, 011°29′52.3″E	722	5	6
MoW	03°52′46.7″N, 011°29′53.8″E	726	2.5	5
NkS	03°53′15.5″N, 011°29′45.3″E	762	8	15
NkW	03°53′13.1″N, 011°29′52.2″E	756	3	5
MeS	03°51′53.1″N, 011°30′08.1″E	731	0[c]	3.5
MeW	03°51′52.9″N, 011°30′06.6″E	733	0	8
MlS	03°51′58.2″N, 011°29′27.4″E	724	1	8
MlW	03°51′56.1″N, 011°29′35.9″E	747	1	3
EtS	03°51′27.7″N, 011°29′04.9″E	727	0	5
EtW	03°51′36.6″N, 011°29′07.7″E	727	0	16
ObS	03°51′32.2″N, 011°29′25.2″E	721	9	5
ObW	03°51′30.7″N, 011°29′18.8″E	715	3	5

[a]0 means that muddy soils are less than 1 m from the wells or springs.
[b]DM-W/S: Distance between muddy soils and wells or springs.
[c]DM-T: Distance between muddy soils and toilets (m).

was covered by a rectangle of cellophane soaked in glycerol, the sample was spread out between the slide and the cellophane to form a smear.

Formalin-ether concentration technique

For the formalin-ether concentration technique, 5 mL of the sample pellet, 2 mL of 10% formalin and 3 mL of ether were introduced into a 10 mL test tube. Four layers were observed in the test tube after homogenisation. The fat layer (debris) was removed with a stick, and the sediment was resuspended by inverting the tube with a quick motion. Finally, two or three drops of Lugol's iodine were added to the suspension which was used for observation and enumeration after mounting between the slide and cover slip. Kato-Katz and formalin-ether techniques were used for the observation of eggs and larvae of helminths.

Identification and enumeration of parasitic elements

The parasitic elements were identified on the basis of their morphology using the identification keys of the World Health Organization (WHO 1994). The (X) number of parasitic elements in 1 L of sample was found starting with the following formula (Ajeagah *et al.* 2010):

$$x = y\frac{V_x}{V_y}$$

where, Vx = pellet volume of 1 L of sample, Vy = pellet volume considered in the identification; y = a number of cysts, eggs and larvae counted in Vy.

Observations were made using an Olympus CK2 inverted microscope at a magnification of 40× for protozoa and 100× for helminths.

Statistical analysis

The graphs were prepared using MS Excel 2010. The average densities of parasitic elements and the standard errors were also determined by the same software. The Bray Curtis index was applied by SPSS to measure the level of affinity between the different sampling points that have been considered in our investigations.

RESULTS

Flagellated enteric protozoa

Total abundances of the cysts of the protozoa for the period of study in muds

Throughout the duration of the study, the abundance of the flagellated enteric protozoa cysts varied from 2,521 cysts for *Retortamonas intestinalis* to 8,192 cysts for *Chilomastix mesnili*. The values of the cysts of *Giardia intestinalis* and *Enteromonas hominis*, respectively, were 6,481 and 2,588 cysts (Figure 2).

Seasonal variation of cysts' abundance in mud

The mud samples in the surroundings of wells and springs near the dwelling houses revealed contamination of all the intake points (100%) considered in our investigation. In general, the highest densities of cysts were recorded during the SRS and in all the samples of mud collected except that of Nkomkana in the SDS.

Seasonal variation and diversity of the densities of cysts in mud

As a whole, the densities of protozoa cysts were higher in the SRS (Figure 3; Figure 4(a)) than in the SDS (Figure 4(b)), except at the sampling points MoW, NkS, M1 W and EtS. Muddy soils in the surroundings of Etoug-Ébé well (SRS) and spring (SDS) were distinguished from the others by their more significant density. An absence of cysts in the mud samples was also observed at NkW in the SDS. The cysts of *Giardia intestinalis* and *Chilomastix mesnili* are represented at all sampling points.

Intestinal eggs and/or larvae of the helminths

Partial variation of the density of the intestinal eggs and larvae of the helminths in muds

The densities of the dissemination forms of helminths in muddy soils around springs and in soils around the wells

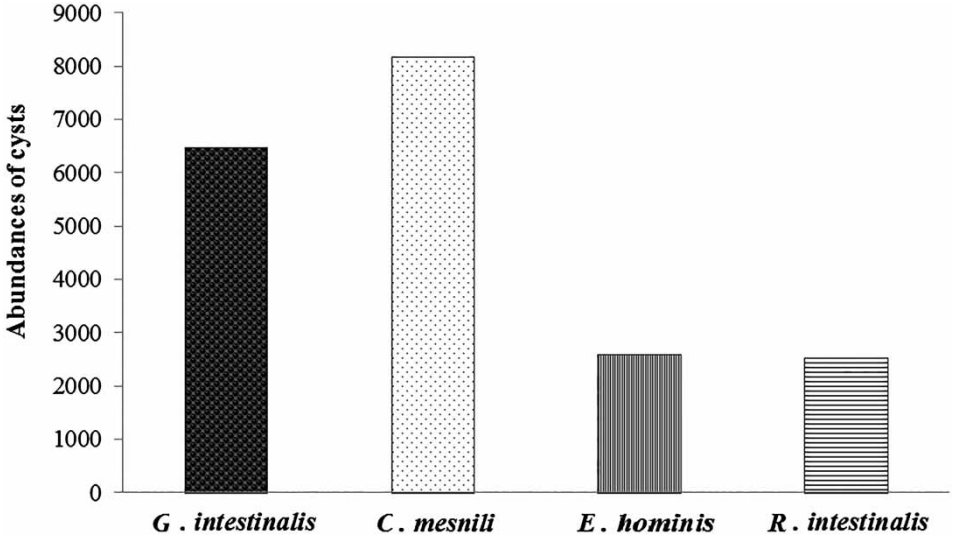

Figure 2 | Total abundance of the cysts of the flagellated enteric protozoa in muds.

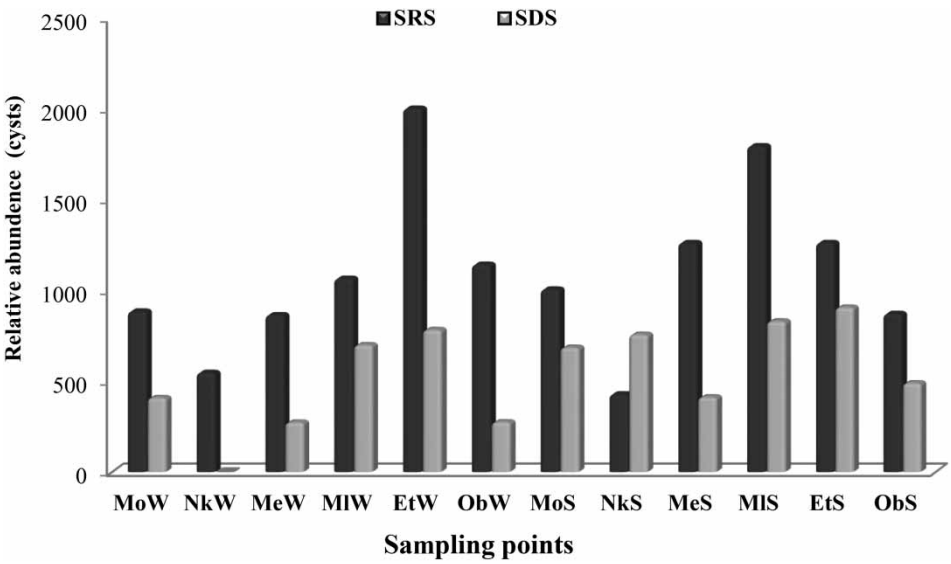

Figure 3 | Partial and seasonal variation of relative abundance of the cysts in muds for the study period.

vary from one point to another. Overall, the highest densities were obtained in the mud samples from the surroundings of the springs compared to those from the surroundings of the wells. Soils at the various points harbour the same species of helminth. The environmental forms of the helminths were found in all muds from the surroundings of the wells and springs. The minimum density of eggs and larvae of helminths was obtained in the muds at NkS (54 agents/L), and the maximum density in Melen 1(274 agents/L) (Figure 5).

Spatiotemporal variation of the densities of dissemination forms of the helminths in the muds

The minimum density of eggs and larvae of helminths are obtained from the muddy soils of NkS during the SDS

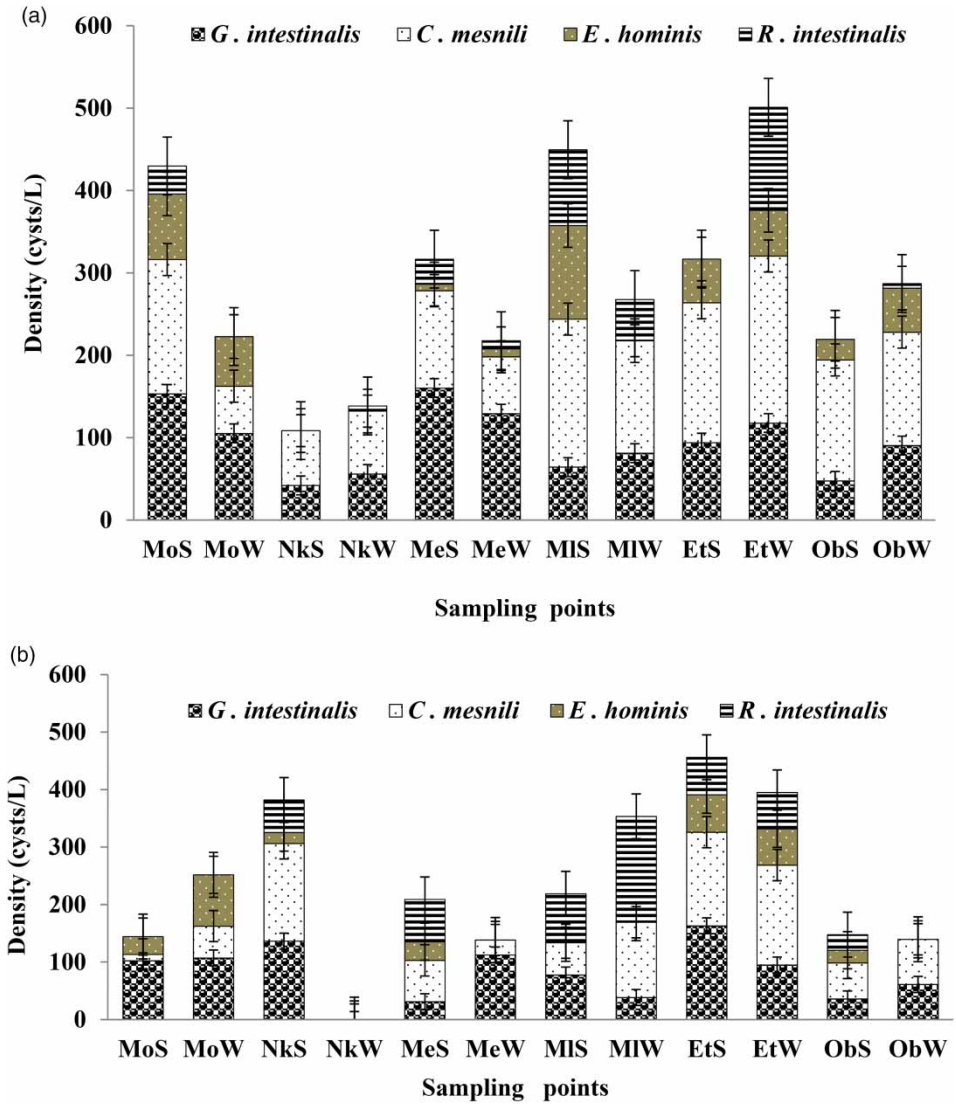

Figure 4 | Spatiotemporal variation of the average densities of the cysts per season: (a) (SRS), (b) (SDS).

(43 ± 16 agents/L) and the maximum density in the muddy soils from MeS during the SRS (333 ± 69 parasitic elements of helminths/L). At all the points, the highest densities of eggs and larvae of helminths in muds are obtained during the SRS (Figure 6).

Parasite infections

The presence of protozoa and helminth in mud suggests the role of mud in the dissemination of pathogens. The abundance of identified parasites is presented for each species. The high concentrations of protozoa and helminths in mud are responsible for waterborne disease (Table 2).

Similarities between the sampling points based on parasites

The similarities between the sampling points based on the biological variation are presented in a dendrogram (Figure 7). They were assessed by the application of the Bray–Curtis dissimilarity index, which is a statistical tool used to quantify the compositional dissimilarity between

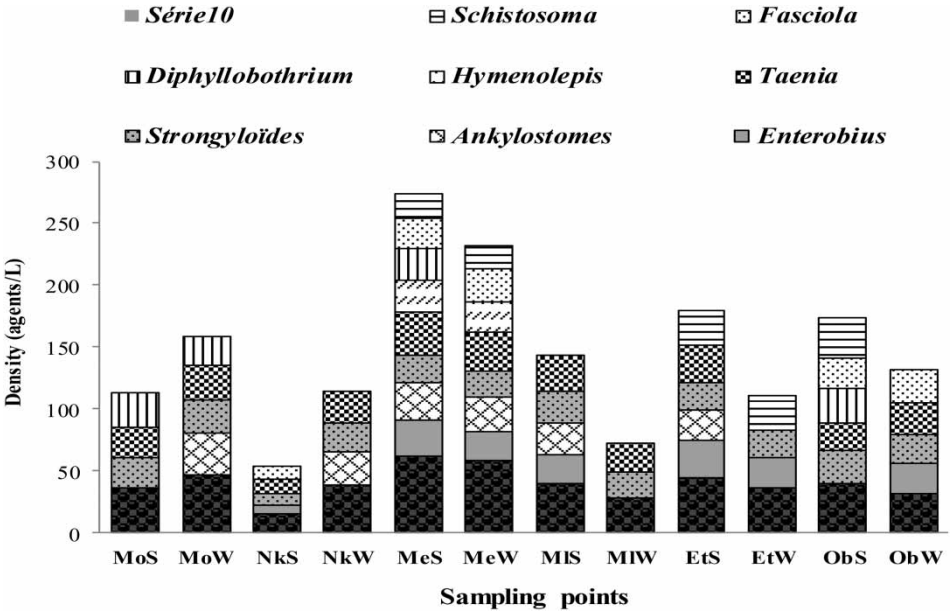

Figure 5 | Spatial variation of densities of eggs and larvae.

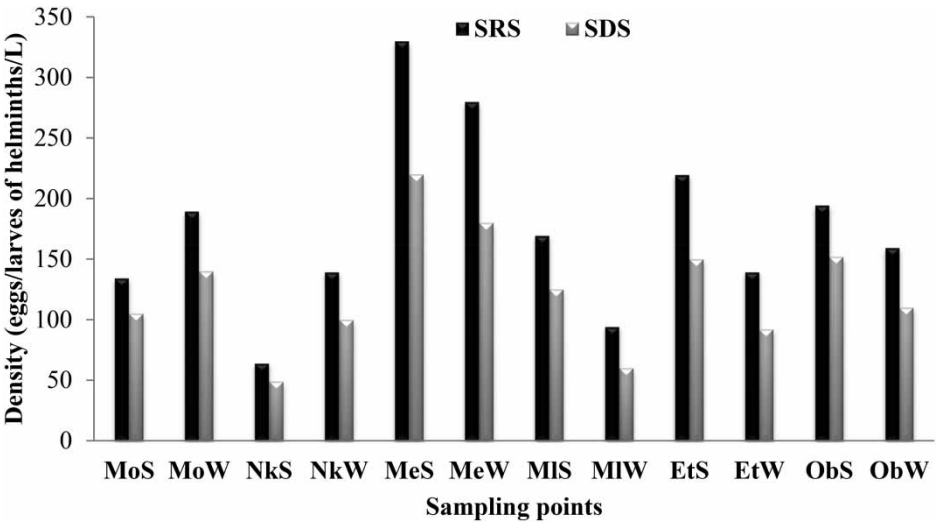

Figure 6 | Spatiotemporal variation of the averages of the densities of intestinal eggs and/or larvae of the helminths.

two different sampling points, based on the counts of the cysts and eggs/larvae identified. It shows 80% similarity between points EtS, EtW, MlS MlW, MoS and ObW which share the same branch and directly receive piggery effluent. Points MeW and NkS show 50% similarity. Points MeS, ObS, NkW and MoW show 50% similarity, representing low parasite contamination.

DISCUSSION

These results indicate the presence of cysts, eggs and larvae in these study areas revealing, according to WHO (2012), contamination of faecal origin and would expose the inhabitants to the risks of contamination if the areas were not sanitized. According to Bloomfield & Nath (2009),

Table 2 | Overall number of parasitic forms (cysts, eggs and larvae)

Group	Species	Abundance (% of total)	Disease
Protozoa	*Giardia intestinalis*	6,481 (25.39%)	Giardiasis
	Chilomastix mesnili	8,192 (32.09%)	Chilomastidiasis[a]
	Enteromonas hominis	2,588 (10.14%)	Non-pathogenic
	Retortamonas intestinalis	2,521 (9.87%)	Non-pathogenic
Helminths	*Ascaris* sp.	1,532 (6.00%)	Ascariasis
	Enterobius sp.	532 (2.08%)	Enterobiosis
	Ancylostoma duodenale and/or *Necator americanus*	558 (2.19%)	Ancylostomosis
	Strongyloides sp.	882 (3.45%)	Strongyloidiosis
	Taenia sp.	948 (3.71%)	Taeniasis
	Hymenolepis sp.	165 (0.65%)	Hymenolepiosis
	Diphyllobothrium sp.	351 (1.37%)	Diphyllobothriosis
	Fasciola sp.	369 (1.45%)	Fascioliasis
	Schistosoma sp.	411 (1.61%)	Schistosomiasis
Total		**25,530**	

[a]Pathogenicity uncertain.

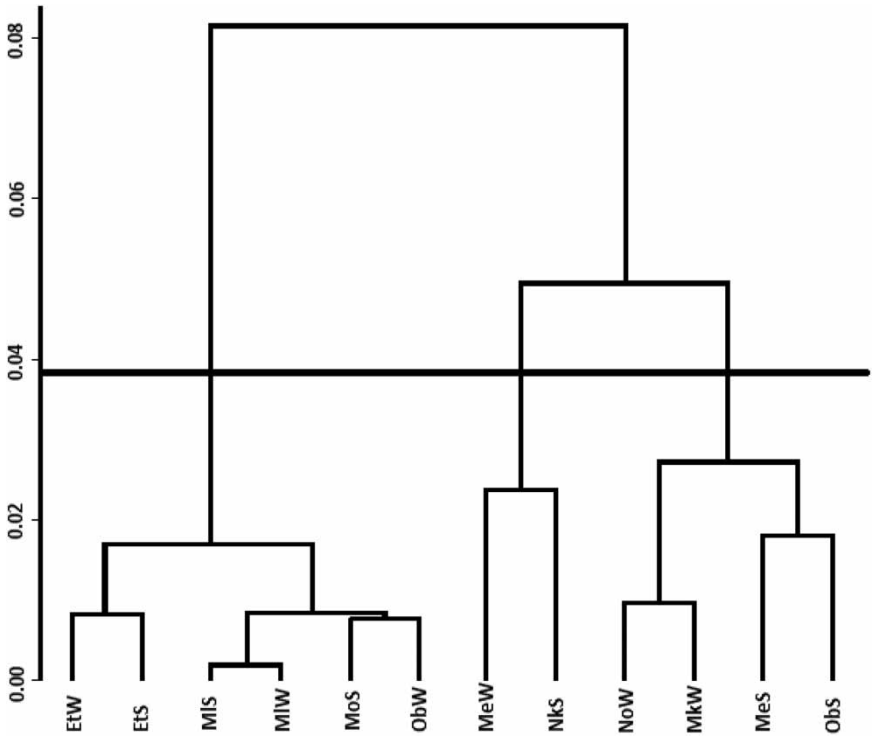

Figure 7 | Dendrogram showing the similarities between the points.

microbiological contamination of muds or soils is widespread due to large-scale practice of open defecation.

Concerning protozoa, the total abundance of the four species of protozoa studied was 19,782 cysts found in muddy soils greater than that of groundwater (14,037 cysts) in the same locality (Ajeagah *et al.* 2016), proving that protozoa cysts were more important in the muddy soils around the wells and springs. This can be interpreted

as showing that the dissemination of cysts is more important in muddy soils than in groundwater. The highest densities of cysts were recorded during the SRS and in all the samples of muddy soils collected except that of Nkomkana in the SDS. This can be explained by the fact that muddy soils are the major recipients of pollutants through draining water which plays a major role in the distribution of resistant forms of the pathogens in the environment. In this regard, Bertrand *et al.* (2004) show that the percentages of contamination of mud by cysts, eggs and larvae are always between 37% and 100% and Hanus (2005) confirmed that muddy soils around water points would worsen the risks of pollution of the wells. This is also applicable to unprotected springs and other water bodies in local communities.

The high density observed in muddy soils at Etoug-Ébé during the study period would be due to a favourable topography that is related to a low sanitation conditions. An absence of cysts in the mud samples has also been observed in Nkomkana well in the SDS. The cysts of *Giardia intestinalis* and *Chilomastix mesnili* are represented at all the intake points. This could be explained by the influence of the size of the cysts, coupled with the thickness of the wall. In fact, the large cysts of the flagellated enteric protozoa (8–17 μm) and (5–10 μm) for *Giardia intestinalis* and *Chilomastix mesnili*, respectively, would resist better in a disturbed environment than the small cysts such as *Enteromonas hominis* (5–7 μm) and *Retortamonas intestinalis* (4–6 μm). In the same way, the work of Ajeagah *et al.* (2016) on groundwater treated with sodium hypochlorite has shown the greater resistance of large flagellated enteric protozoa (*Giardia* and *Chilomastix*) rather than small cysts (*Enteromonas* and *Retortamonas*).

The biological analysis of mud samples also reveals the presence of helminth eggs and larvae such as *Ascaris* sp., *Enterobius* sp., Ankylostomes (*Necator* sp. and/or *Ancylostoma* sp.), *Strongyloides* sp., *Taenia* sp., *Hyménolepis* sp., *Diphyllobothrium* sp., *Fasciola* sp. and *Schistosoma* sp. According to Bouhoum *et al.* (1997), the pathogenic organisms present in the muddy areas of the community reflect its health state. Indeed, the number and the varieties of parasites found in muddy soils are related to the level of infestation of the human population and/or animal population present. These parasites are excreted in the external area in the form of eggs or larvae with the faeces of its host during open faecal defecation. The eggs of *Ascaris* sp. are highly represented among the helminth eggs in this study. In this situation, WHO (1989) mentioned that the eggs of *Ascaris* are resistant in the environment and considered as indices of parasitoses and poor sanitation conditions. The densities of the dissemination forms of helminths in muddy soils around the springs and wells vary from one point to another. The maximum density of eggs and larvae of helminths in the muds around springs were obtained at Melen 1 (274 agents/L) while the minimum density was recorded in the muddy soils around the Nkomkana spring (55 agents/L). This could be explained by the unhealthy behaviours that the populations close to the muddy points adopt. These muddy soils could also constitute a source of pollution for water in the wells and springs in the SRS. For this reason, Kassim (2005) affirms that waste water forms pools during the rainy season and from time to time overflows into the water collection point, which could be a spring or well or tap water.

With regard to these results, it is well accepted that muddy soil can become contaminated with protozoa and helminths in high concentrations, and that it can act as a vehicle and source of the spread of intestinal diseases (Bloomfield & Nath 2009). The presence of various pathogens in our environment can lead to polyinfection and cause more health disorders. The infection of humans and animals by the same parasite is called zoonosis. Protozoa and helminths are among the most common causes of infection and disease in humans and other animals. The diseases have a major public health and socio-economic impact. Indeed children between 5 and 16 years would be most exposed to these sources of contamination due to the defects of hygienic conditions and the negligence of the community when fetching water from wells and springs, uncontrolled playing of the children around these water points and absence of a collection system, treatment and water drainage. It is therefore necessary to improve the water supply and sanitation facilities. According to Hutton *et al.* (2007), improved water supply generally involves better physical access and the protection of water sources, including standpipes, boreholes, protected springs or wells or collected rainwater, and improved sanitation generally involves physically closer facilities, less waiting time and safer disposal of

excreta, including septic tank, simple pit latrine or ventilated improved pit latrine. In cases of contamination of muddy soils, the risk of epidemic is not to be excluded at the family level or beyond in the event of the consumption of water around muddy soils. The distribution of environmental forms of protozoa and helminths vary from one point to another according to the level of contamination (Figure 7).

It would also be crucial to mention that these muds could support contamination of the water table by the process of infiltration or draining water into unprotected wells and springs and also of streams via domestic effluents. In this regard, Nanfack *et al.* (2014) and Ajeagah *et al.* (2010) demonstrate that contamination of wells and springs would be related to the sources of pollution in the neighbourhood. It can also be related to domestic effluents, cattle ranching and lack of toilet facilities. These results show that not only are soils reservoirs of the cysts, eggs and larvae, but can also be a crucial factor of contamination of groundwater. In all, the high level of soil contamination constitutes a potential source of parasitic infection and suggests that the treatment and disposal of human and animal excreta continues to be a public health concern in our urban municipalities (Moura *et al.* 2010).

CONCLUSION

This study showed that soils around water points are reservoirs for the dissemination of the environmental forms of protozoa and helminths. The analysis of the results reveals that muds shelter the cysts of flagellated enteric protozoa (*Giardia intestinalis, Chilomastix mesnili, Enteromonas hominis* and *Retortamonas intestinalis*) and the eggs and/or larvae of the helminths (*Ascaris* sp., *Enterobius* sp., *Necator americanus* and/or *Ancylostoma duodenale*, *Strongyloides* sp., *Taenia* sp., *Hymenolepis* sp., *Diphyllobothrium* sp., *Fasciola* sp. and *Schistosoma* sp.) revealing a contamination of faecal origin. Overall, the dissemination forms were commoner in the SRS than in the SDS due to the impact of drainage and infiltration of water charged with faecal matter. The contamination of soils in proximity to dwelling places can be a factor in groundwater contamination and would expose the households to health risks. Given the results of this research we recommend that the population conduct sustainable water management, to cleanse and treat the surrounding soils of springs, wells and water points, to practice good rules of hygiene, to plan water drainage systems and to adopt an eco-citizen approach.

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