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Baseline Study on the Occurrence of Cryptosporidium Spp from Streams Water, after Torrential Rains in Bamenda, Cameroon

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Abstract
Oocycts of Cryptosporidia are zoonotic infective stages of *Cryptosporidia spp* that can easily contaminate the environment if not properly disposed of. Torrential rains would usually wash them into the nearby streams and likely be consumed by humans when the water is used. Cryotosporidiosis in people with suppressed immune systems have no cure as the robust oocycst are not destroyed by chlorination. The aim of this study is to determine the presence of Cryptosporia oocycts in stream water after torrential rains 60 samples of stream water were collected immediately after rain storm in June and July 2014, from 5 streams flowing through Bamenda town. 2 samples of 1.5L were collected from each stream, on 6 different days. *Cryptosporidia ssp* were present in all the streams but there was no significant difference between their occurrence and the stream's location, Chi square = 2.605, >0.05The streams flowing through Bamenda town are polluted
Keywords: storm-water, zoonotic, Cryptosporidiosis, chlorination, oocysts

1. 1NTRIODUCTION

Zoonotic pathogens of enteric origin are likely sources of contamination of the environment, with transmission via soil, agriculture, water and sediments (Bonetta *et al.,* 2011). Proceedings of the meeting entitled "*Cryptosporidium*: from Molecules to Disease" (2001) observed that there are many reports of this parasite being acquired from public water supplies. However, these may well represent cases of cryptosporidiosis transmitted to humans by domestic animals such as kittens and puppies, from ruminants on farms, or by contact with other humans. Food grown on soil fertilized with manure could also be considered a potential source of infection; however, contaminated water represents the major source of infections for humans. *Cryptosporidium* oocysts may remain viable in water for over 140 days (Hooda, *et al,* 2000) and are very resistant to the most common disinfectants (Campbell *et al,* 1982) making them difficult to destroy by conventional chlorination.

The public health importance of cryptosporidiosis has not been widely reported despite the growing interest of the slang "water is life" as there is no on-going monitoring of our water sources for contaminants. The Bamenda highlands is actually a water shed and our water is not safe as it is likely being contaminated with environmentally resistant Oocysts of *Cryptosporidia spp*. The method use in purifying our water(Chlorination) does not eliminated these Oocysts, and their infective dose is very low as only 1 to 10 Oocysts(Pereira *et al,* 2000) can elicit an infection (cryptosporidiosis) which has adverse effects on people with weakened immune systems like HIV/AIDS patients, children, the elderly pregnant women and people on chemotherapy. At the moment in Bamenda the only dam supplying the Bamenda metropolis has no water and denizens move out of town to get water.

Cattle and sheep are gazing free range in this water catchment and during torrential rains, all cattle dung and sheep dropping are washed into the dam. Zero grazing is not practiced. To better understand the gravity of the contamination of our water, it is necessary to analyze faecal samples from animals grazing in water catchment areas as well as the water taking its rise from the water catchment area. Rural to urban migration is putting a lot of pressure on the scarce portable water. Some residents who have built closed to stream in areas that are not accessible have built their toilets close to stream. Pipes are connected to the toilets and when there are heavy rains, they will open pipes to let out faeces into the nearby streams figure 2Some gardeners down stream use this water to irrigate their vegetables. Previous studies in Cameroon by Ajeagah *et al*, (2007) and

Ajeagah, *et al, (2005*) identified Cryptosporidia Oocysts in municipal lake in Yaounde-Cameroon. There have been a lot of studies on the biodynamic of the resistant forms of these emerging pathogens in water (Smith and Thompson, (2001), Ajeagah, *et al*., 2005). Most of these analyses have been carried out on streams not on humans. Ajeagah, *et al.,* (2005) asserted that quantitative data on the identification of Oocysts in lake ecosystems and their survival in a lentic environment is still unavailable in developing countries in general and Cameroon in particular. The hydrosystem of the municipal lake in Yaounde was considered as a significant reservoir of Oocysts, that may be resuspended in the water column and give rise to sporadic increases in the Oocysts in the whole river basin in which is situated the lake, resulting in the outbreaks of Cryptosporidiosis as many gardeners along the streams emanating from this lake use the water for irrigation**.**

The main objective of this study was to identify Oocyst of *Cryptosporidium ssp* in stream water collected after rainstorm since the method that is used in disinfection of water cannot eliminate this parasite. Storm-water is not being harvested for potable and non-potable reuse applications across in Bamenda, but the water sediments when it reaches the flood plains, Fig.1. The water is used where there are pools for swimming, washing of clothes, watering of gardens, cleans of floors. Some of the storm water flows into the water bodies in Bamenda up station which is treated as portable water. We determined whether our water is contaminated or not using storm-water because it will likely have parasites from the torrential rains. The rationale is that once Oocysts are identified then human infections are very likely. There is paucity of data for pathogen from storm-water in Cameroon

2. MATERIALS AND METHODS

2.1 Study area:

The Study Area Bamenda in the **Bamenda Grassfields**. The **Western High Plateau**, **Western Highlands**, or **Bamenda Grassfields** is a region of Cameroon characterised by high relief, cool temperatures, heavy rainfall, and savanna vegetation.

The area experiences two major seasons: A long, wet season of nine months, and a short, dry season of three months. Average rainfall per year ranges from 1,000 mm to 2,000 mm. The Western High Plateau was once heavily forested. However, repeated cutting and burning by human beings has forced the forest back to areas along the waterways and has allowed grasslands to expand into the area. Sudan savanna forms the dominant vegetation. This consists of grassfields—leading to the name *Bamenda grassfields* around the city of Bamenda—and short shrubs and trees that shed their foliage during the dry season as a defence against brush fires and dry weather. Farming is done along the streams or swamp rice in showing the plains. Where there are gardens, they are irrigated with the water.

The study areas is Bamenda situated between longitude $10^{0.}08'$ to $10^{0}.12'$ E and latitude $5^{0}.55'$ to $6^{0}00'$ N. Bamenda covers a surface area of 71.23 square kilometers. Bamenda is the regional head quarters of the North West Region of Cameroon.

Urbanization has had severe impacts on the hydrology of Bamenda through the creation of roads, settlements and the generation and the dumping of wastes in river and stream. Three broad agricultural land use systems – crop based farming, pure pastoralism and mixed crop-livestock – are practiced in the study area. In these systems, traditional farming practices are a combination of crop and small livestock husbandry (pigs, goats, sheep, rabbits) for the native crop farmers, cattle and limited crop production for Mbororo/Hausa/Fulani non-natives, and pure crop and/or livestock farms for commercial urban dwellers. In the dry season, water is scarce and people have to make do with the highly polluted streams. Children swim in pools of water in the flood plains, with inherent danger in the accumulated pathogens.

2.1.1 Morphology of Bamenda

The morphology of Bamenda is characterised by a gentle sloping Up-station area separated from an undulating to flat Down Town area by an escarpment which is about 7 km long with trend $N37^{0}$ and about 150 m high. The climate is the humid tropical highland type characterized by two seasons a rainy season and a dry season. The vegetation is characterised by short stunted tress that is the savannah type. River Mezam is the main river which drains Bamenda. The hydrography presents a characteristic dendritic drainage pattern

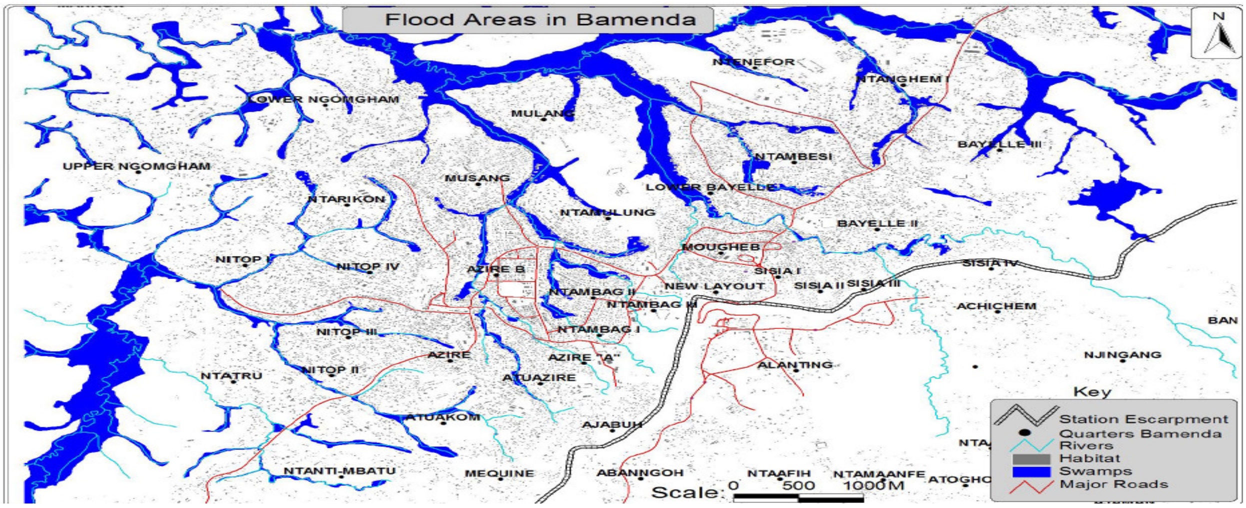

Figure 1. Dendretic network of streams from the Bamenda watershed supplying Bamenda Town
Source: **Tita *et al.*** Syllabus Review, Sci. Ser. **3 (201**2): 1 – 10.

Farming is the main activity carried out here which is mostly crop based farming, pure pastoralism and mixed crop livestock. This study will provide adequate information that the government should use to protect and advice the inhabitants on water use and prevention of waterborne infections. The Bamenda Area constitutes a major watershed which has been very useful to the local population as they depend on it in the pursuit of their developmental activities (water supply, agriculture, grazing and fishing). Overgrazing, the seasonality of streams and scarcity of potable water in the dry season are just part of the chain of problems originating from the degradation of the watershed due to population pressure. Kometa and Ebot (2012) observed that Watersheds are generally considered as points of development especially in countries which rely on water dependent activities. A major problem confronting watersheds remains the increase in human population and land use mutations, which sets in to degrade watersheds. The implications on communities concerned are usually grave – the decline in water quality and quantity which translates into the upsurge of water borne diseases and a drop in agricultural production. Most of local streams Down-town lack clearly defined head streams but the others from Up-station reach the foothill zone through a series of small waterfalls over bare hill slopes which, together with the hills, form the most striking feature of the Bamenda urban area. Since the streams are youthful, flow is rapid. Thus, debris and watershed runoff from the slope sub-system become part of the input of the stream channels. During the rainy season, the linear-dendritic streaming system (Figure 1), combined with various erosional processes such as river valleys, causes spillage, subjecting houses and properties, particularly those along stream courses, to periodic flooding during torrential rainstorms. It is during this rainstorm that some inhabitants near the streams open their toilets to discharge faeces into the streams.

Figure 2.0. Pipes projecting from a toilet into a nearby stream in New layout Nkwen. Picture by Polycarp Ndikvu July 2015

(Figure 2) and thick film, of stool settles after the rains (figure 3).

Figure 3.0. Film on the water below the pipes projecting from the toilet into the stream in New layout Nkwen. Picture by Polycarp Ndikvu, July 2015

Pig styles are also built along the stream banks and when rains fall the faeces are washed into the stream water.

Figure 4. Pigsty the along New Layout stream. Picture by Polycarp Ndikvu July 2015

Some gardeners use the water down stream to irrigate their vegetables (figure 5)

Garden along the Ayaba stream, Picture by Polycarp Ndikvu July 2015

2.1.3 Sample collection

A reconnaissance visit was made to human-water points along each of the streams flowing through Bamenda Metropolis, a week before sampling started**.**

Stream water was collected in duplicates of 1.5L sterile containers, after torrential rains from 5 streams running through Bamenda town. The stream water was collected immediately after 6 different storm events. The samples were transported immediately to the Phyto-Biotechnology Research Foundation (PRF) laboratory for analysis. A total of 60 samples were collected for the study

2.1.4 Laboratory analysis

Water samples were filtered using Sieve (strainer) with small holes, of 400–450µm in size. The small inexpensive nylon tea or coffee strainer was used to remove large particles. The method used to recovered oocysts and/or cysts was formol-ether concentration method as per (Ukaga *et al*., 2002; Cheesbrough 2006).

A small amount of about 750 µl of the concentrated suspension of each water source was drawn out. 2 smears were made on slides, air dried and fixed with methanol (Kinyoun) and the other observed under X10 and X40 (wet smear (Cheesbrough, 2009). Lugol's iodine was added for the identification cysts and ova.

The smears were stained with Modified Kinyoun's Acid-fast Stain (Cold) as described by Garcia, (2001)

Microscopic identification of the parasites in each was under magnification 10X and 40X objectives. Standard textbooks and pictures provided (Lindsay *et al.,* 2000) by the Phyto-Biotechnology Research Foundation Institute Bamenda, Cameroon, were used to established the identity of the parasites.

2.1.5 Statistical analysis

Data was entered into Ms Excel® 2003 (Microsoft corporation, USA) and analysis were conducted using SPSS for Windows version 12. Prevalence was calculated as a percentage of d/n where d is the number of animals infected and n = Total number of water samples analysed. Chi square test was used to explore the association between the occurrence of Cryptosporidia oocysts in a stream and the stream's location. Also a p- value of <0.05 was considered to be statistically significant.

3. RESULTS

Of the 60 samples collected from the 5 streams, 12 samples were collected from each stream. The prevalence *Cryptosporidium spp* and *Giardia spp* was determined according to the different streams sampled. Giardia spp was included only because of its zoonotic potentials.

The occurrence of *Cryptosporidium spp* in Sisia Stream 3(25%)table 1a and 1b, New layout stream 4(33.33%) table 2a and 2b, Progressive Comprehensive high School stream(PCHS) 3(25%)table 3a and 3b, Ayaba stream 1(25%) table 4a and 4band Lourdes stream 4(33%)table 5a and 5b. New Layout stream was highly polluted 75% while Ayaba stream was the least polluted 25% table 4a. table 6 show the overall prevalence in all the streams.

The distribution of crytosporodium across sample1 is the same as the distribution across sample 2 implying that the results for occurrence of cryptosporidium is not affected by the sample the collected.(results cannot be biased by sample). There is no significant association between the occurrence of Cryptosporidia oocysts in a stream and its location, Chi square = 2.605, >0.05

Table 1. Occurrence of parasites in Sisia stream

DAY	*Cryptosporidium ssp*	*Giardia ssp*
1	1	0
2	0	1
3	1	0
4	0	0
5	1	0
6	0	0
Total occurrence	3	1

Legend: 1= occurrence; 0 = absences

Table 1b. Prevalence of parasites in Sisia stream

Parasite	**Occurrence**	**Prevalence (%)**	**Overall Prevalence (%)**
Cryptosporidium spp	3	25	33.33%
Giardia spp	1	8.33	

Table 2. Occurrence of parasites in New Layout stream

DAY	*Cryptosporidium ssp*	*Giardia ssp*
1	0	1
2	1	0
3	1	1
4	1	1
5	1	1
6	0	1
Total occurrence	4	5

Table 2b Prevalence of parasites in PCHS stream

Parasite	**Occurrence**	**Prevalence (%)**	**Overall Prevalence (%)**
Cryptosporidium spp	4	33.33	75%
Giardia spp	5	41.67	

Table 3a. Occurrence of parasites in PCHS stream

DAY	*Cryptosporidium ssp*	*Giardia ssp*
1	0	1
2	0	0
3	1	1
4	0	1
5	1	1
6	1	0
Total occurrence	3	4

Table 3b Prevalence of parasites in PCHS stream

Parasite	**Occurrence**	**Prevalence (%)**	**Overall Prevalence (%)**
Cryptosporidium spp	3	25	58
Giardia spp	4	33	

Table 4. Occurrence of parasites in Ayaba stream

DAY	*Cryptosporidium ssp*	*Giardia ssp*
1	0	0
2	0	0
3	0	0
4	1	0
5	0	0
6	0	0
Total occurrence		

Table 4b Prevalence of parasites in Ayaba stream

Parasite	**Occurrence**	**Prevalence (%)**	**Overall Prevalence (%)**
Cryptosporidium spp	1	25	8.33%
Giardia spp	0	0	

Table 5.Occurrence of parasites in Lourdes Stream

DAY	*Cryptosporidium ssp*	*Giardia ssp*
1	1	0
2	1	0
3	1	1
4	0	0
5	1	1
6	0	1
Total occurrence		

Table 5b Prevalence of parasites in Lourdes stream

Parasite	**Occurrence**	**Prevalence (%)**	**Overall Prevalence (%)**
Cryptosporidium spp	4	33	57
Giardia spp	3	25	

Table .6 Occurrences and Prevalence of parasites in streams in Bamenda Metropolis

***Cryptosporidium spp* /*Giardia spp* positivity in Stream water in BAMENDA Town**					
DAYS OF SAMPLE COLLECTION	SISIA STREAM	NEW LAYOUT STREAM	PCHS STREAM	AYABA STREAM	LOURDES STREAM
D1	1/0	0/1	0/1	0/0	1/0
D2	0/1	1/0	0/0	0/0	1/0
D3	1/0	1/1	1/1	0/0	1/1
D4	0/0	1/1	0/1	1/0	0/0
D5	1/0	1/1	1/1	0/0	1/1
D6	0/0	0/1	1/0	0/0	0/1
SUB-TOTAL	3/1	4/5	3/4	1/0	4/3
PREVALENCE	25%/8.33%	33.33%/41.67%	25%/33%	25%/0.0%	33%/25%
TOTAL	33.33%	75%	58%	8.33%	57%

Legend: The first figure in each stream represents sample 1 as well as *Cryptosporidium spp* while the second figure represents sample 2 as well as *Giardia spp*. 1= occurrence, 0= absence

4. DISCUSSION /CONCLUSION

Epidemiological study carried out by Ntonifor *et al,* (2013) to determine the prevalence, intensity of infection gastrointestinal (GIT) parasites in grazing ruminants in Jakiri, Bui Division, North West Region of Cameroon, did not consider Cryptosporidiosis as being of any importance. Previous studies in Cameroon by Ajeagah *et al*, (2007) and Ajeagah, *et al, (2005*) identified Cryptosporidia Oocysts in municipal lake in Yaounde-Cameroon. However, the source of contamination was never traced. The present study is to determine the presence of oocysts in stream water and if the location of the streams has an influence in their occurrence; or the distribution of oocyst in the first sample is different from the second sample.

In conclusion there is urgent need fro an integrated approach to the detection of *Cryptosporidia spp* by veterinarians, molecular epidemiologists, and parasitologists in order to establish the dynamics of this parasite in Cameroon as this would lead to better understanding of zoonotic potentials of this parasite.

4.1 Recommendation:

All the flood plains in town could be reclaimed converted into public gardens and parks. Sanitary inspectors should move along the streams everyday to see that the people should not build along the streams or through sewage.

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