

ASSESSMENT OF CRYPTOSPORIDIUM INFECTION IN BENAKUMA HEALTH DISTRICT OF THE NORTH WEST REGION OF CAMEROON

Polycarp N. Chia^{*1}, Kenneth A. Yongabi², Fon E. Tata³, Mary-Teresia Ghumbemsitia⁴, Priscillia Ngie Nde Tuma⁵, Pius M. Tih⁶, Yongbang V.Yuh⁷ & Bertrand W. Maimo⁸

¹Biomedical Laboratory Scientist, lecturer/HOD, Department of Biochemistry Catholic University of Cameroon (CATUC) Bamenda - Cameroon. PO Box, 782 Mankon Bamenda, Northwest Region Cameroon. Website: www.catuc.orgpolycarc@gmail.com.

²Proprietor, Phyto-Biotechnology Research Foundation Institute, PO Box, 921 Bamenda North West Region-Cameroon Director of Research Catholic University of Cameroon (CATUC) Bamenda

³Biomedical Laboratory Scientist, lecturer, Department of Biochemistry Catholic University of Cameroon (CATUC) Bamenda - Cameroon. PO Box, 782 Mankon Bamenda, Northwest Region Cameroon. Website: www.catuc.org.

⁴Assistant Lecturer Department of Mathematics, Catholic University of Cameroon (Catuc), Bamenda.

⁵Assistant Lecturer Department of Microbiology, Catholic University Of Cameroon (Catuc) Bamenda.

⁶Professor of Public Health Administration, Director of Health Services, Cameroon Baptist Convention Health Board, Bamenda, North West Region; Cameroon, Website: www.cbchealthservices.org

⁷Physician, Faculty of Medicine and Biomedical Sciences, University Of Yaoundé

⁸Department of Biochemistry, Faculty of Science, Catholic University of Cameroon (CATUC) Bamenda, Cameroon. P.O Box, 782 Mankon- Bamenda

Abstract

Keywords:

Benakuma; Prevalence; Cryptosporidium; oocysts. Bio sand filters.

This study was designed to investigate the distribution of *Cryptosporidium* and associated risk factors amongst the denizens of Benakuma, a rural agrarian community, in February 2017. Stool samples from 524 participants were assessed for *Cryptosporidium* oocysts. A questionnaire on risk factors was issued by oral interview. The stools were analyzed by standard methods (normal saline, Lugol's iodine preparation and formol ether concentration methods and modified Kinyoun Ziehl Neelson). The prevalence rate was 72(27.5%). Prevalence of infection with respect to age also showed a statistically significant difference ($p < 0.05$), with the highest rate in those aged between 5-15 years, and the least in those aged between 0-5 (0%). The prevalence rate of infection was highest 4(4.4%) in participants with no formal education, and lowest in literate participants. Method of hand washing and use of soap and/or detergent had no effect on prevalence of infection. There was a significant difference in the prevalence of *Cryptosporidium* relative to types and number of animal with which they came in contact. Age, use of soap/detergent, income, education levels, and animal waste disposal methods had strong associations ($P < 0.05$) with the prevalence of *Cryptosporidium* in Benakuma.

Introduction

Cryptosporidiosis is a zoonotic disease that is acquired due to environmental exposure to oocysts. There have been important cases of *Cryptosporidiosis* outbreaks; in Carrollton, Georgia (LeChevallier *et al.*, 1991) and Milwaukee, Wisconsin, (Mackenzie *et al.*, 1994) where indicator organisms were absent altogether. *Cryptosporidiosis* is a notifiable human disease in New Zealand (Cowie *et al.*, 2013) and higher rates of infections are in rural cases both in developed and in developing countries. From epidemiologic studies, Current and Garcia (1991); confirmed the assertion that *Cryptosporidium* is more prevalent in developing countries (5% to >10%) than in developed countries (<1% to 3%). This is to be expected as most developing nations are incapable of enforcing strict hygienic standards.

1.1 Significance

Diarrheal diseases are considered as one of the health problems worldwide and *Cryptosporidium* is one of the etiologic agents that has no cure in people with altered immune systems like HIV/AIDS patients, children, pregnant women and the elderly. One of the most reared ruminants (cattle) in the North West Region of Cameroon harbors *C. parvum* which has zoonotic potentials (Chia *et al.*, 2015).

It is a common enteric pathogen in humans and domestic animals (Nichols *et al.*, 2010; WHO, 1992; Tchuenté *et al.*, 2009) worldwide with a very low infective dose of one to ten sporulated oocysts which are immediately infectious when excreted in faeces. Nichols *et al.*, 2010), Chalmers *et al.* (2010) and McDonald *et al.* (2010) have conclusively demonstrated the risk posed by *Cryptosporidium* in untreated or inadequately treated drinking water contaminated by waste from human and/or animal bowels.

A baseline study in Cameroon was done by Dennis *et al.* (2011) to determine the prevalence of waterborne protozoan parasites (*E. histolytica/dispar*, *G. lamblia*, and *C. parvum*) in two rural agrarian villages (Nloh and Bawa) in the West Region of Cameroon. These communities had a history of frequent diarrhea, which was linked to faecally contaminated water. Nloh had a prevalence of 25.9%, and Bawa, a prevalence of 8.8%. The main reason attributed to this difference in prevalence was reported by Richardson *et al.* (2011) who posited that the provision of clean drinking water in Bawa, by use of Bio-Sand Filters (BSFs), along with implementation of an education program addressing basic matters of sanitation and hygiene that started in the summer of 2006 a year preceding the protozoological survey, could have likely done the trick. When used according to the manufacturers, the BSF has been reported to remove 90%–99% of waterborne pathogens as recently indicated by Stauber *et al.* (2009), who detected that the use of BSFs reduced the probability of diarrheal disease by 47% among households in Bonaoa, Dominican Republic. Data from Bawa, Cameroon reveals an 84% reduction of diarrheal disease, and a 65% reduction of the occurrence of typhoid, following the implementation of BSFs along with a program of health education on sanitation and hygiene (Damon Callahan, 2010: unpublished thesis).

There is enough conclusive evidence both published and unpublished, that the BSF is efficient in removing waterborne pathogens as well as its effectiveness in reducing diarrheal disease. No studies have been conducted that compare the actual prevalence of waterborne parasites before and after implementation of water improvement systems using BSFs in Cameroon.

Cryptosporidiosis is self-limiting in immunocompetent people, and healthy carriers can act as a reservoir host. The robust infective stage oocysts are not destroyed by the commonly used chlorine used to disinfect water. There is paucity of information on the dynamics of this parasite. A lot of domestic animals are kept which are likely sources of infections. These animals especially cattle are grazing at free range in water catchment areas. Hygiene is poor and sewage management exposes human to contamination.

The aim of this study was to assess the prevalence of *Cryptosporidium* in Benakuma Health District a rural agrarian community in the Northwest Region of Cameroon.; with no pipe borne water.

1.2 Cross Sectional Study

This is a baseline study in a rural area of North West Cameroon that involved the collection of stool from consenting individuals and issuing of questionnaires to participants of all ages and its analysis according to standard protocols.

1 teaspoon full of 524 stools was collected and processed immediately in the field, according to the protocol (Macroscopy, saline and Lugol's iodine wet mount) in the makeshift laboratory in the health fair round. The remaining stools were stored in a cool box for 12 or 24 hours prior to processing in the Catholic University of Cameroon (CATUC) laboratory by the formal ether concentration method and Kinyoun Carboll Fuschin staining. The slides were examined by 2 Laboratorians by 10X, 10X and 100X objectives and standard charts from CDC used for confirmation.

1.3 Justification

Due to the outbreak of cryptosporidiosis in North West Wales, Chris Lines, (2005) performed microbiological investigations using environmental samples and demonstrated the same strain of *Cryptosporidium hominis* in pre- and post-treatment water samples. Therefore, water, even after standard treatment, cannot be guaranteed to be free from cryptosporidium oocysts even in Europe, what of Africa.

The coccidians are important emerging pathogens. They are capable of producing disease in immunocompetent and immunocompromised hosts. It is in this second category of patients that the coccidians causes its greatest significance (Ribes *et al.*, 2004). Many physicians remain unaware of their clinical importance, however tests for these protozoans are not routinely requested in our health institutions.

This study will bring into focus its public health significance in Cameroon. In addition, the results of this research may draw the attention of local health practitioners to take this parasite into consideration in laboratory diagnosis and as well as health education to avoid infection. Management systems of ruminants should be modified to avoid contamination of the humans and/or environment. This portable water scenario (Figure 1) necessitated this study to be carried out in Menchum Division. Chlorination is commonly practiced in Cameroon for the treatment of water, but chlorine at consumable concentration in water does not destroy *Cryptosporidium* oocysts.

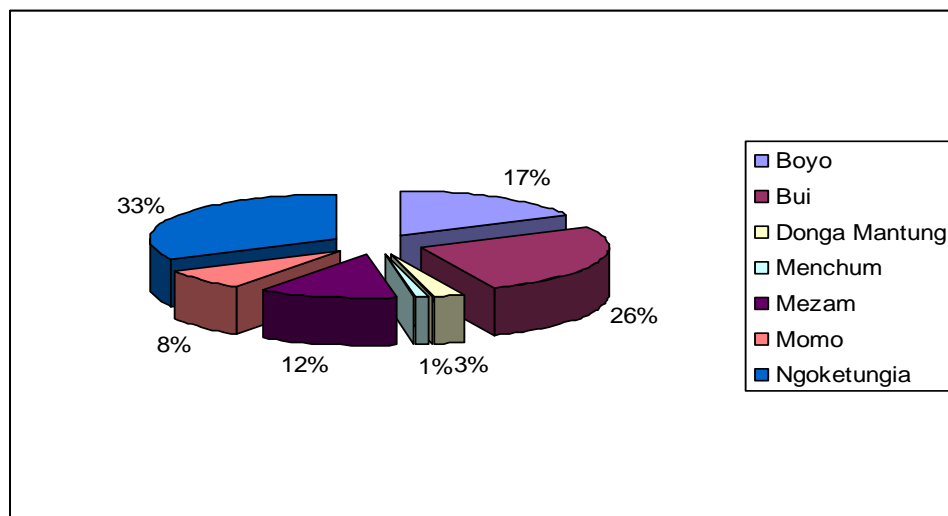


Figure 1. Connectivity of the Divisions of the North West Region by Portable water

Figure 1 indicates that the Division in the North West region with the lowest connectivity by portable water is Menchum Division while the Division with the highest household connectivity is Ngoketungia (Annual reports of Divisional delegations of Ministry of Water and Energy 2005).



Figure 2: Cattle drink from the same source with humans in Benakuma, Menchum Division.

Figure 2.below show cattle drinking from the same source with human. Water for human consumption is harvested from the same water point where cattle freely drink. People living downstream also drink the water untreated or not boiled. (Picture by Polycarp N Chia 2016)

Materials and methods

2.1 Study Area

The area of study, the N. W. Region of Cameroon lies between latitudes $5^{\circ} 45'$ and $9^{\circ} 9'$ N longitudes $9^{\circ} 13'$ and $11^{\circ} 13'E$. It covers an area of about 17400 km² and it is bordered in the North and West by the Republic of Nigeria, on the South by the West and South West Regions and in the East by the Adamawa Region. Administratively the North West Region is divided into seven Divisions namely: Boyo, Bui, Donga Mantung, Menchum, Mezam, Momo and Ngoketunjia respectively with the following administrative capitals: Fundong, Kumbo, Nkambe, Wum, Bamenda, Mbengwi and Ndop. Bamenda is the headquarters of the North West Region (map of the NWR showing study site).

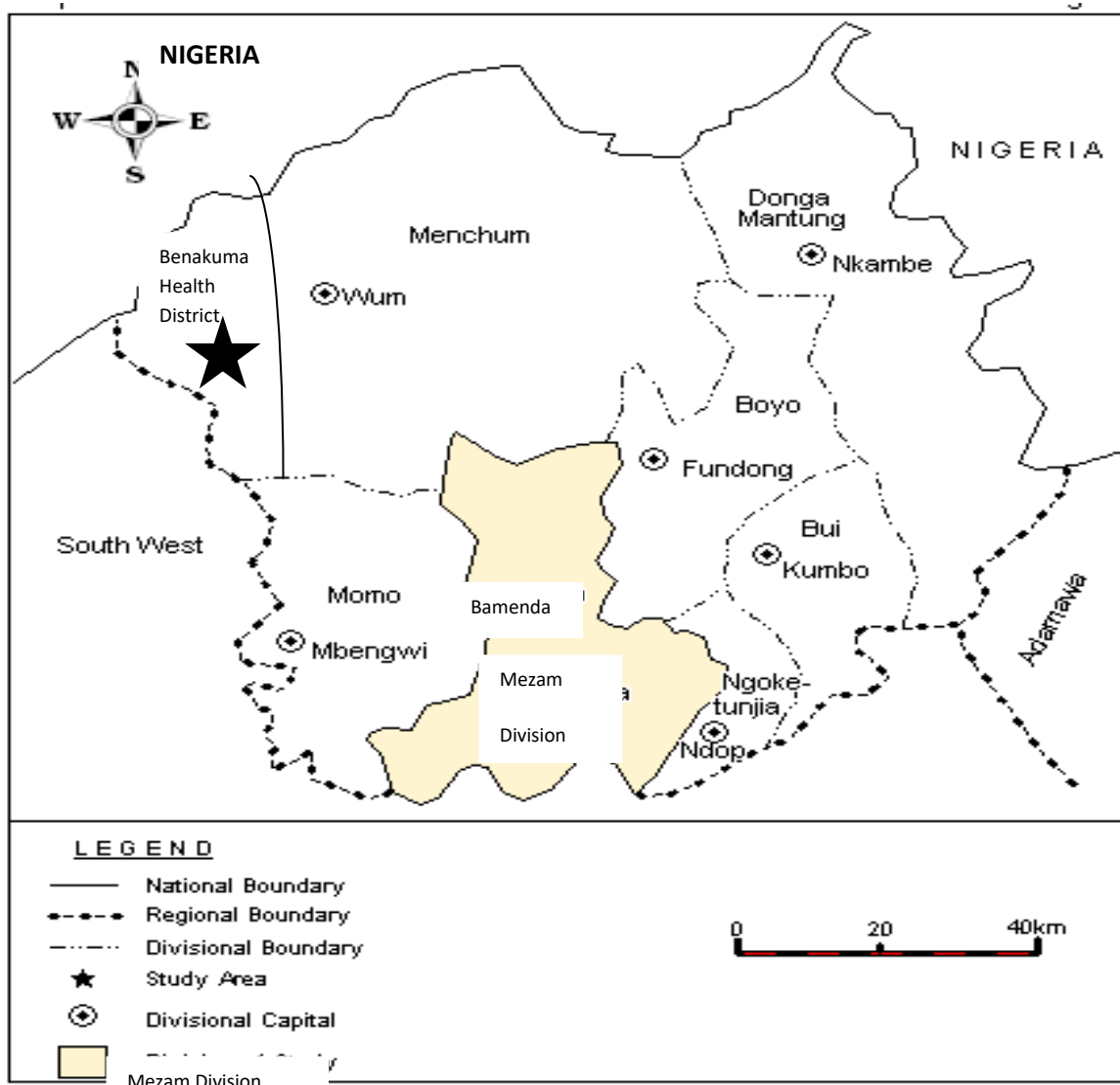


Fig.3. Map of the North West region showing the Benakuma in Menchum Division

Source: Adapted from Bamenda Urban Council 2009.

The study site was Benakuma Health District in Menchum Division with a population of 55,700 inhabitants, made up of the following rural communities: Befang, Modelle, Okoro, Mujang, Beba, Batomou, Benabinge, Benado, Benakuma and Bawuro, a neighbouring tribe to the Republic of Nigeria. This Health District is enclaved and 80Km from the Regional Head Quarter but can only be reached by 4-wheel drive vehicles in 3hours.

This study took place during a health fair organized by the Cameroon Baptist Convention Health Board (CBCHB) in the Benakuma Health District. The communities had been mobilized by a team of field health workers of the CBCHB, the District Medical officer for Benakuma and the community leaders (Chiefs, Parliamentarian, District officer, counsellors), who were in all in attendance during the health fair days.

2.2 Ethical Consideration

Ethical approval for this study (Ethical clearance number IRB2014-33,) was provided by the Ethical Committee, Institutional Review Board of the CBCHB (Chairperson Nancy Palmer, PhD.) Written informed Consent to carry out this work was rigorous and was sought on the spot from the participants and from parents of minors after explaining the purpose and objective of the study. Those who could neither read nor write English or French were assisted using the local Pidgin English. The children whose parents did not consent were excluded, in accordance with the Declaration of Helsinki of 2008 on ethical principles for medical research involving human Subjects.

2.3 Faecal Sample collection/Questionnaire

All participants who consented were given a wide mouth sterile universal bottle, to collect about a teaspoon-full of their stool in nearby toilet, without contaminating with urine. It was only after stool was produced that the sample bottles were labelled and the pre-tested questionnaire administered. The questionnaire was issued to each individual (by oral interview) to obtain information on the following Socioeconomic data: age, sex, methods of hand washing, before meals and after toilet use, animal contact both at home and the community, type of toilet use, water contact activities, buying and eating of fruits along the road, source of drinking water, drinking water away from home, eating of earth. Those who were unable to produce their samples immediately were asked to bring the stool passed first in the morning of the second day.

2.4 Sample Examination

Both saline wet mount and formol ether concentration techniques were used for examination of intestinal parasites as described by the WHO (1991). Only the wet mount was done immediately in the health fair ground in a makeshift laboratory. The rest of the samples were stored in a cool box and taken to the Laboratory of PRF at the end of the second day for concentrated and stained the third day. The Modified Kinyoun's Acid-Fast Stain (Cold) was used as per Garcia, (2001) and modified by Cheesbrough, (2009), for detection of *Cryptosporidium* oocysts. The slides were examined by 2 Laboratorians by 10X, 10X and 100X objectives and standard charts from CDC used for confirmation.

2.5 Statistical Analysis

The data obtained from the study was analysed using SPSS version 21.0 for Windows (SPSS, Chicago, IL, USA) software for analysis and Excel. Pearson's chi-square test was used to determine the statistical significance between the dependent variable and independent variables. A confidence interval (CI) of 95% was considered ($\alpha = 0.05$). Available results of the laboratory analysis are presented as frequency tables with percentages, histograms and pie charts.

Results

In this survey, five hundred and twenty-four (524) consented participants produced stool samples and responded to the pre-tested and modified questionnaire. There were 359 females and 165 males and their stools were examined for *Cryptosporidium* oocysts by standard methods.

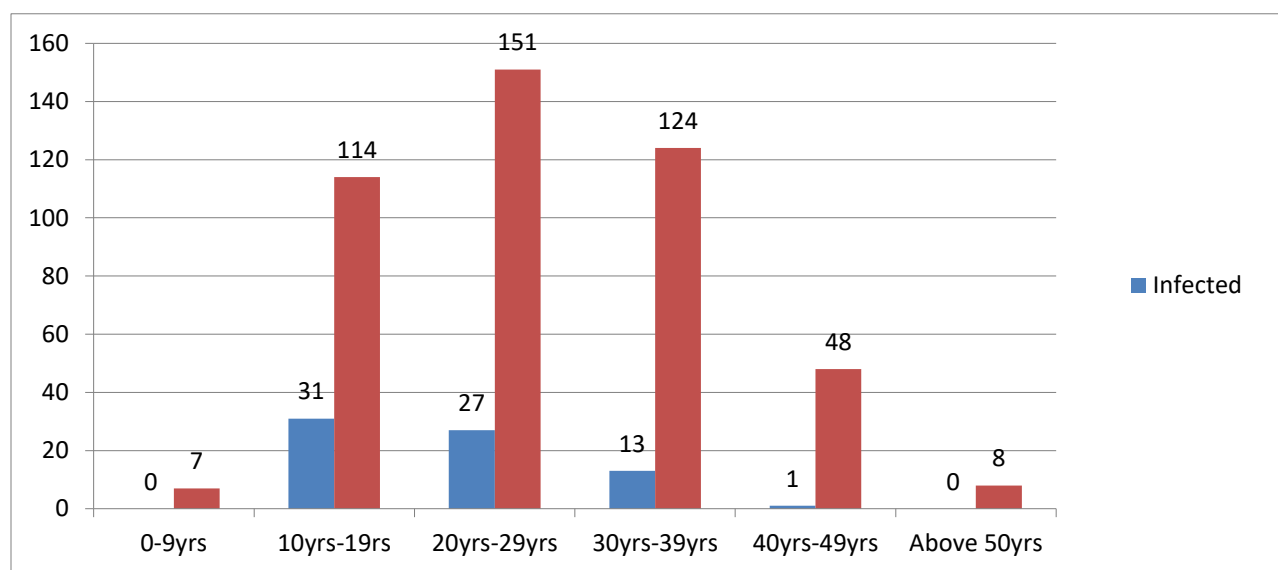
The overall prevalence of infection with *Cryptosporidium* oocysts was 72(13.7%). The rate of infection in females 49(68.50%) was higher than in males 23(31.50%) (Table 1.0).

Table 1.0: Gender related prevalence of Cryptosporidium oocysts.

Sex	Number examined	Number positive(%72)
Males	165	23(31.5)
Females	359	49(68.5)
Total	524	72(100)

The highest prevalence rate 31 (21.4%) was in those age 10-19 years and the lowest (0%) was in participants aged 0-9 years and above 66 years, respectively. A trend was observed with a decrease prevalence of infection as the age brackets of participants' increase.

Statistically, there was a significant association between the different age groups and the prevalence of *Cryptosporidium* oocysts infections ($P=0.004$) as shown on figure 4.



($p=0.04$)

Figure 4: Age related Prevalence of *Cryptosporidium* oocysts Infection (n=524)

Out of the 524 studied participants who provided stool, the highest prevalence of *Cryptosporidium* oocysts was in student 30(41.7%) followed by farmers 24(33.3%) and those with no occupation. 18(25%). The least was in civilservants 0%. There was a very strong association between occupation and prevalence of infection with *Cryptosporidium* oocysts ($p=0.001$).

Table 2: Occupation related *Cryptosporidium* oocysts infection.

Occupation	Number examined	Number infected(%72)
students	218	30(41.7)
Farmer	230	24(33.3)
Civil servants	16	0(0)
No occupation	60	18(25)
Total	524	72(100)

Prevalence of *Cryptosporidium* infection with respect to level of education showed the highest prevalence 40(18.2%) in participants with primary school education and followed by participants with secondary school education 28(15.4%). The lowest prevalence was in those who had no formal education. Participants who had post graduate education had no infections (0%). A decrease of prevalence of infection was observed with increase in level of education. Statistically, there was a very strong association between the level of education and the prevalence of *Cryptosporidium* oocysts infection ($p=.008$).

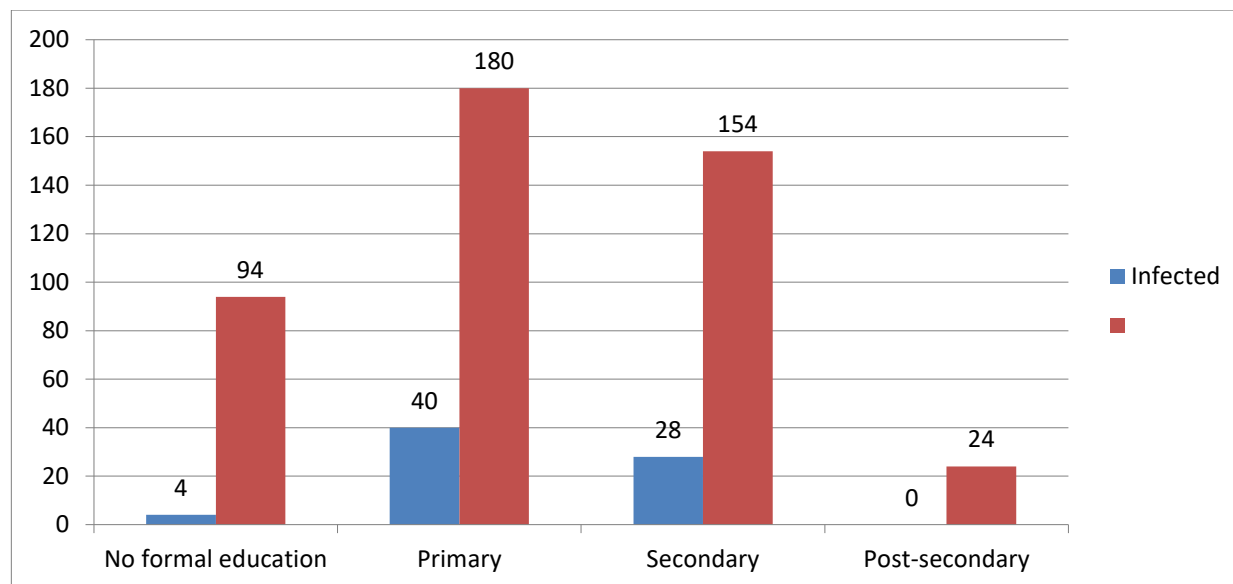


Figure 5: Education related *Cryptosporidium* oocysts infection.

A total of 524 participants who consented to this study responded to the item of method of disposing of faeces. Out of the 72 respondents who were positive for *Cryptosporidium* oocysts, majority, 64(88.9%) used Pit latrines, while 8(11.1%) used the bush. Respondents who used flush toilets were not infected 0%. Statistically, there was a significant association between the type of toilet used and the prevalence of infections with *Cryptosporidium* oocysts ($p=0.04$).

Table 3: Mode of Human bowel waste disposal related *Cryptosporidium* oocysts infection

Mode of human waste disposal	Number examined	Number infected(%72)
Bush, potty or the grounds	20	8(11.1)
Pit latrine	496	64(88.9)
Flush	8	0(0)
Total	524	72(100)

($p=0.04$)

Out of the 72 participants who were positive for *Cryptosporidium* oocysts, the highest prevalence 38(52.8%) was in those who did not wash their hands with soap before handling food, followed by 18(25%) who always washed their hands before handling food. The lowest prevalence 16(22.2%) was in those who did not always wash their hands before handling food with soap. There was a statistically significant association between hand washing practice before handling food and the prevalence of *Cryptosporidium* oocysts infections.

Table 4: Hand washing practice with soap related *Cryptosporidium* oocysts infection

Hand washing practice with soap before handling food	Number examined	Number infected(%72)
Yes	158	18(25)
No	256	38(52.8)
Not always	110	16(22.2)
Total	524	72(100)

Out of the 72 participants who were positive for *Cryptosporidium* oocysts, 46(63.9%) washed their hands by pouring water before handling food, while 26(36.1%) washed their hands with water in a container before handling

food. Statistically, there was a strong association between hand washing methods before handling food and prevalence of *Cryptosporidium* oocysts infection ($p=0.02$).

Table 5: Hand washing method related *Cryptosporidium* oocysts infection

Hand washing method before handling food	Number examined	Number infected(%72)
Washing under running tap water or pouring	392	46(63.9)
Washing using water in a container	132	26(36.1)
Total	524	72(100)

Discussion

Jeevitha *et al.* (2014) acknowledged that roughly half of the world's population lives under the conditions that generate nutritional stress and parasitic diseases with protozoan parasites or helminthes especially in third world countries. Intestinal protozoan parasitic infections have been known to cause public health problems, especially in rural areas where there is inadequate water supply, poor personal hygiene and nutritional deficiencies (Babatunde *et al.*, 2013). Ill health due to intestinalhelminthes infection is said to be preventable through regulardeworming (DeSilva *et al.*, 2003). As a consequence, the World Health Assemblyurged the international community to take firm action againstSTH infections in 2001. Since then, the 'Partners forParasite Control' (WHO member states, the World Food Program,UNICEF, the World Bank, research institutions, and non-governmentalorganizations) set out to deworm 650 million school-aged childrenat risk of STHs and schistosomiasis regularly by 2010 (WHO, 2002). There is a growing awareness of the intolerable burden intestinal parasitosis pose due to the 'so-called' neglected tropical diseases (Hotez *et al.*, 2006). There is no documented literature on *Cryptosporidiosis* in the North West Region of Cameroon.

The purpose of this study was the assessment of the parasitic protozoan *Cryptosporidium* in denizens of Benakuma Health district of the North West Region of Cameroon. Determining the prevalence of this protozoan in this community was also important to assess the risk factors that predisposed the community to the infection. The cycling of pathogens can occur between humans and also between animals and humans (Hunter and Thompson, 2005, Feng, *et al.*, 2011, Fuchslin *et al.*, 2012).

The overall prevalence of infection with *Cryptosporidium* oocysts was 72(13.7%). Previous studies by Dennis *et al.* (2011) in a rural agrarian village in the West Region of Cameroon, observed a prevalence of 3.6% *C. parvum*. This could have been due to the small sample size of 85 participants as compared to the present study. Also, the significantly lower prevalence of protozoal infection could have been due to the provision of clean drinking water by use of Bio sand filters(BSFs), and education program on sanitation and hygiene that began a year before the protozoological survey (Richardson *et al.*, 2011).

This data is very significant as there is no existing documented data concerning the prevalence of waterborne protozoan parasites in the human population of the North West Region of Cameroon especially *Cryptosporidia* species. This results show that *Cryptosporidiosis* is present in Benakuma Health District of the North West Region of Cameroon, a group of rural agrarian villages. Human diarrhoeal epidemics have occurred in many cattle raring regions of Cameroon (Djomassi *et al.*, 2013) but each time methods of diagnosing causative agent does not include *Cryptosporidium* but mostly cholera.

1. Relative to gender

The rate of infection in females 49(68.50%) was higher than in males 23(31.50%). The observed prevalence in Benakuma is similar to those of Dozie *et al.* (2011), who observed lower prevalence in males (26.7%) than females (32.5%) in rural areas of Imo State in Nigeria.

Mbanugo and Agu (2006) and Chukwuocha *et al.*(2009) asserted that females are usually more infected than men in rural communities, probably because women spend more time on farmland cultivating vegetables, washing food in

streams. They also do most of the house chores using water from the nearby stream like cleaning the floor, pit toilets, potty or stool of children or animals. They carry animal waste to the farms to use as fertilizers, especially cattle dung (Chia *et al.*, 2015) that may contain *Cryptosporidium* oocysts. All these activities increase their chances of infection. From these results women living in the Benakuma Health district are exposed to unhygienic conditions. However, the results of this study are not in support of Dennis *et al.* (2011), who did not observe any significant differences in the prevalence of cryptosporidiosis between genders in the village in the Western Region of Cameroon, where both men and women are actively engaged in farm work.

Except for *Cryptosporidium* oocysts, no other parasites were found in the stool of the participants, this could have been due to the mass deworming and distribution of ivermectin by the Ministry of Public Health of Cameroon a month preceding the study. Ivermectin is a broad-spectrum antiparasitic agent used in the treatment of onchocerciasis, strongyloidiasis, ascariasis trichuriasis, filariasis and enterobiasis (Hamilton *et al.*, 2014; Mehlhorn and Heinz, 2008).

The sources of drinking water in Benakuma health district include streams, ponds (used in the dry season especially), boreholes, springs, bottled water and pipe borne water; which are untreated. *Cryptosporidium* and *Giardia* may be introduced into water-bodies by point or nonpoint (diffuse) pollution sources (Erin *et al.*, 2014). Anti-helminthics are readily available in Benakuma as ambulant drug vendors abound.

In Cameroon, focus is mainly on deworming activities which have been increased to encompass all ten regions since 2007 as a result of a co-ordinated effort of the Ministry of Public Health and the Ministry of Basic Education with national and international partners (Tchuenté and N'Goran, 2009). Some helminthes like *Strongyloides* and *Taenia* have been reported to be resistant to anti-helminthics (Utzing and Keiser, 2008).

Cryptosporidia infection in local communities in the North West of Cameroon has not been established as infections of healthy adults are self-limiting; however, such individuals can act as a source of infection to individuals with suppressed immune systems. Due to the invasive nature of this *Cryptosporidium* it has caused numerous deaths in immunocompromised patients due to unresolved diarrhoea as most of the people will seek traditional treatment and the real cause of their ailments especially HIV will not be diagnosed and untreated. However, we never ruled out HIV/AIDS in our study population. Cryptosporidiosis is a major cause of diarrhoea in children with and without human immunodeficiency virus (HIV) infection in developing countries (Peng *et al.*, 2003; Tumwine *et al.*, 2005). In these countries, cryptosporidial infection in early childhood has been reported to be associated with subsequent impairment in growth, physical fitness, and cognitive function (Niehaus *et al.*, 2002). In India, *Cryptosporidium* spp. is a leading cause of infectious diarrhoea in children, with reported positivity rates ranging from 1.1% to 18.9% (Kaur *et al.*, 2002). Chronic cryptosporidiosis may be complicated by biliary tract disease, malabsorption, and death in individuals with AIDS and malnourished children (Pantenburg *et al.*, 2008). These results contradicted the reports of higher prevalence in males than females by other researchers (Egberongbe *et al.*, 2010). From the present results women living in Benakuma Health district are exposed to unhygienic conditions. However, the results of this study are not in support of Dennis *et al.* (2011), who did not observe any significant differences in prevalence cryptosporidiosis between genders in the village in West Region of Cameroon. This should be expected because in the West region of Cameroon (Francophone) men and women work on the farm on daily bases as compared to Benakuma where most of the farm work is done by women.

2. Relative to age

The highest prevalence of infection was found in the children aged between 10-19years, 31%, this may be attributed to the primary and secondary school going age where they spend most of their time away from home. School premises have very low levels of hygiene as animals are usually seen wandering and children picking food that had fallen on the ground and eating. Participants in 20-29 age brackets had the next highest prevalence of infection. This could have been because those actively involved in farming are found in this group. Exposure to animal waste could have attributed to their infection. Children aged between 0-9years in rural communities, do not attend school and their parents take care of them. This report collaborates with previous observation made in this regard by Egberongbe *et al.* (2010). A trend was observed in which as the ages of the participants increased there was a

reduction in the prevalence of infection. This could have been because as the individuals age they observe more hygiene and sanitation. There was a very high association between age brackets and prevalence of infection with *Cryptosporidium* oocysts. Statistically, there was significance association between the different age groups and the prevalence of *Cryptosporidium* oocysts infections ($p=0.004$).

However, the results of this study are not in support of Dennis *et al.* (2011), who did not observe any significant differences in prevalence cryptosporidiosis among age groups in the village in Western Region of Cameroon. Local communities had good water supply due to the use of BSFs and education program on sanitation and hygiene that began a year prior to the study (Richardson *et al.*, 2011).

3. Relative to occupation

This study has documented the highest prevalence 30(41.7%) of *Cryptosporidium* oocysts in students, followed by farmers 24(33.3%) and those with no occupation 18(25%). The least was in civil servants 0%. In rural communities students 30(25%) work along with their parents on family land in crop production, so they would be exposed to infections as well as those who are farmers 24 (33%) especially as the use of untreated animal manure is common, hence the high infection rate. The participants who had no occupation 18 (25%) were likely drop out from schools who were either sex workers or park boys in Benakuma town which served as a transit point for traders to Nigeria. They were less exposed to sources of infections as compared to farmers. The civil servants were least infected 0% as they were likely not engaged in farming and their educational level must have enlightened them to practice good hygiene and sanitation like hand hygiene and sewage disposal. These infections are likely to be linked to the everyday activities of the individuals rather than gender (Jeevitha *et al.*, 2014).

4. Relative to education level

Prevalence of *Cryptosporidium* oocysts with respect to level of education showed the highest prevalence 40(18.2%) in participants with primary school level of education, and followed by those with secondary school level of education, while those with post-secondary school levels of education had the lowest prevalence (0%). These results are in line with those of Dibua *et al.* (2013) who suggested that people with lower levels of education are more likely to eat food and drink water from questionable sources as they carry out their work. Hence a trend was observed in which the prevalence of infection reduced with increase in the educational level of the participants. Those with no formal education showed a very low prevalence (4%) of infection, which implies that lack of formal education does not necessarily translate to ignorance about good hygiene and sanitation practice.

5. Prevalence of *Cryptosporidium* oocysts relative to the mode of human bowel waste disposal.

The present study documented a high prevalence 64(88.9%) of *Cryptosporidium* oocysts in those who used Pit latrines and the lowest in those using flush toilets 8(11.1%). This can be attributed to the fact that those who used flush toilets had access to water and soap to wash their hands (Birnbaum, 2003, McGeer, 2007)) and the hands were washed under running water. While those who used pit toilets did not. The participants, who used the grounds or bushes to empty their bowels, also had no water and/or washed their hands with water in a container.

6. Prevalence of *Cryptosporidium* oocysts relative to hand-washing habits and methods

Soap suspends easily removable micro-organisms allowing them to be washed off more easily (Birnbaum, 2003). Hand sanitizers are readily available and some people may likely use it (Johnson *et al.*, 2005). This was confirmed by those who did not use soap to wash their hands before handling food as they had the highest prevalence of infection with *Cryptosporidium* oocysts 38(52.8%) those who always washed their hands before handling food had a prevalence of 18(25%), likely because of the wrong method of washing their hands. Those who washed their hands fortuitously with soap had a prevalence of 16(22.2%). Hence, these participants did not have good hand hygiene and were exposed to infections.

Comparing the prevalence rates in participants who observed good hand-washing methods and does who didn't, indicted that the prevalence of *Cryptosporidium* oocysts was lower in those who washed hands under running water consistently. This is because this is a recommended method of washing (Pittet, 2005); as the pathogen are easily

washed off. Those who habitually washed their hands in a pail showed a higher prevalence, which may stem from the fact that washing hands in a pale provides for recontamination, as germs remain in the pail.

Prevalence cryptosporidiosis due to hygienic conditions (hand washing, eating or drinking water out of home, type of toilets) was just an approximation of the truth as participants were very enthusiastic to give even unsolicited information about their neighbors but said “they were not like their neighbors”. Despite the fact that we told them we were not awarding any marks for best hygienic practices to individuals but trying to get the truth in order to propose better ways of living in order to avoid any disease.

Conclusion

This study was an attempt to establish evidence of Cryptosporidium infection in man and animal, in order to ascertain the presence of the zoonosis cycle of the disease in water which is the main vehicle through which the disease is easily spread.

Benakuma is a rural agricultural area and as asserted by Cowie and Bell (2013) is expected to have a high rate of Cryptosporidiosis. However, true prevalence in a community should be higher as out of the population of 55700 only 524 participated in the survey, a sample not too appropriate to make inference from relative to the total population size of the community.

In this study it has been difficult to isolate different potential sources of infection, as most cases who consumed untreated drinking water also had direct contact with animals. However the consumption of untreated drinking water has been shown to be widespread amongst cryptosporidiosis cases in Benakuma and has to be considered a significant potential source of infection. When used the BSF as prescribed by the manufacturer, has been observed to remove 90%–99% of waterborne pathogens (Duke *et al.*, 2006; Stauber *et al.*, 2006; Baumgartner *et al.*, 2007) This data is very significant as there is no existing documented data concerning the prevalence of waterborne protozoan parasites in human population of the North West Region of Cameroon especially Cryptosporidia species. This results show that Cryptosporidiosis is present in Benakuma Health District of the North West Region of Cameroon. Diarrhoeal epidemics have occurred in many cattle rearing regions of Cameroon but each time methods of diagnosing causative agent does not include Cryptosporidium but mostly cholera.

The data demonstrates therefore that Cryptosporidiosis is occupational disease, poor hygiene, ignorance and wrong methods of management of domestic animals, wrong methods of washing hands as well as the age of the individual.

Co-infection

The other parasites that were identified microscopically were *Entamoeba histolytica* (3.5%), *Enteribius vermicularis* (1.23%), *Strongyloides stercoralis* and *Cryptosporidium spp* (27.5%). The overall enteric parasites prevalence of 33.46 % was observed.

Recommendations

The method of treatment of portable water in Cameroon is chlorination hence oocysts in contaminated water cannot be eliminated. Further study is necessary to determine the full significance of the consumption of untreated drinking water on cryptosporidium infection as well as other novel methods of disinfection of water.

Prevention messages through health education regarding reducing transmission during and after contact with animals would likely prove beneficial although difficult to implement. Reduction in the consumption of untreated drinking water may also help reduce the incidence of cryptosporidiosis and other water borne communicable diseases in Benakuma. Frequently used methods of treating portal water like boiling and chlorination alone have been proven to be ineffective in the destruction of the oocysts, so methods like the use of BSFs should imperatively be employed in the water filtration process, especially for domestic use. This study provides the baseline data regarding waterborne protozoan infections from the village of Benakuma that will enable comparisons subsequent to the provision of clean water using BSFs to be made. The money disbursed by the World Bank (2015) to Cameroon

for “Third Phase of the Community Development Program Support Project” should be used in construction of Bio-Sand Filters.

Limitation: prior to this study, we did not separate the population into HIV positive and HIV negative participants. This could have confounded our results.

In subsequent studies there should be a separation of these two groups of participants.

Acknowledgment

We acknowledge the field staff of the Cameroon Baptist convention Health Board, District Medical Officer for Benakuma, the Mayor and chiefs for the mobilisation of their communities. Also, profound gratitude goes to the director of health services of the Cameroon Baptist Convention that modified the questionnaire to include questions about neighbors from consenting participants, and for the genius initiative of the health fare platform. Finally, I acknowledge the Consulting team of the CBCHB, who assisted in sending participants to the location of the laboratory which was in a secluded place for easy consent and privacy. The Catholic University of Cameroon (CATUC), Bamenda PO, BOX 782 Mankon for providing the laboratory space for this work.

Declarations

Funding: NA.

Conflict of interest; the authors declare that no competing interests exist

References

1. Akiyoshi DE, Tumwine JK, Bakeera-Kitaka S, Tzipori S. Subtype analysis of Cryptosporidium isolates from children in Uganda. *Journal of Parasitology*. 2006 Oct;92(5):1097-100.
2. Annual reports of Divisional delegations of Ministry of Water and Energy North West Region of Cameroon 2005
3. Babatunde RO, Omotesho OA, Sholotan OS. Factors influencing food security status of rural farming households in North Central Nigeria. *Agricultural Journal*. 2007;2(3):351-7.
4. Baumgartner J, Murcott S, Ezzati M. Reconsidering ‘appropriate technology’: the effects of operating conditions on the bacterial removal performance of two household drinking water filter systems. *Environmental Research Letters*. 2007 Jun 8;2(2):024003.
5. Birnbaum D, Canadian Committee on Antibiotic Resistance. Antimicrobial resistance: a deadly burden no country can afford to ignore. *Canada communicable disease report= Relevé des maladies transmissibles au Canada*. 2003 Sep 15;29(18):157.
6. Chalmers RM, Robinson G, Elwin K, Hadfield SJ, Thomas E, Watkins J, Casemore D, Kay D. Detection of Cryptosporidium species and sources of contamination with *Cryptosporidium hominis* during a waterborne outbreak in north west Wales. *Journal of water and health*. 2010 Jun 1; 8(2):311-25.
7. Cheesbrough M. *District laboratory practice in tropical countries*. Cambridge university press; 2006 Mar 2.
8. Chris Lines. Outbreak of cryptosporidiosis in North West Wales. Report of the outbreak control team, 2005.
9. Chia, PN, CN Ukaga, KA Yongabi, BEB Nwoke, and PM Tih (September, 2015)-Prevalence of *Cryptosporidium parvum* in Dung collected from cattle in Bamenda, Northwest Region of Cameroon. *Indian Journal of Medical Research and Pharmaceutical Sciences*; 2(9)
10. Cowie G, Bell A. A retrospective review of notified human cryptosporidiosis cases in the Waikato region of New Zealand, 2004 to 2011. *The New Zealand Medical Journal (Online)*. 2013 Sep 27;126(1383)URL: <http://journal.nzma.org.nz/journal/126-1383/5845/>
11. Chukwuocha UM, Ashiegbu KK, Dozie INS, Aguwa OC (2009). The Perspectives of adolescents on common disease and medicines used. A case study of a public secondary Schools in Owerri, Imo State. *Nigeria. Scient. res. Res*. 4(11):1403-1407.

12. Dennis J. Richardson, Katherine R. Richardson, Karen Damon Callahan, Jeanette Gross, Pierre Tsekeng, Blaise Dondji, and Kristen E. Richardson. Geohelminth Infection in Rural Cameroonian Villages. *Comparative Parasitology* Jan 2011 : Vol. 78, Issue 1, pg(s) 161- 179 <https://doi.org/10.1654/4444.1>
13. DeSilva NR, Brooker S, Hotez PJ, Montresor A, Engels D, Savioli L. Soil-transmitted helminth infections: updating the global picture. *Trends in parasitology*. 2003 Dec 31;19 (12):547-51.
14. Dibua, Uju Marie-Esther, Ejere, Vincent, Lijoka, Ilemobayo and Uma Akunnaya(2013) Intestinal parasitic infestations among people living with HIV/AIDS in Nsukka, Southeast Nigeria *Int.J.Curr.Microbiol.App.Sci* 2(11): 539-550.
15. Djomassi LD, Gessner BD, Andze GO, Mballa GE. National surveillance data on the epidemiology of cholera in Cameroon. *Journal of Infectious Diseases*. 2013 Nov 1;208(suppl 1):S92-7.
16. Dozie I, Nkem B, Chukwuocha U. Cryptosporidiosis in Imo State, Nigeria. *Journal of Rural and Tropical Public Health*. 2011;10:106-10.
17. Duke WF, Nordin RN, Baker D, Mazumder A. The use and performance of BioSand filters in the Artibonite Valley of Haiti: a field study of 107 households. *Rural Remote Health*. 2006 Aug 2;6(3):570.
18. Egberongbe HO, Agbolade OM. Cryptosporidiosis among children in relation to toilet facilities and water sources in Ijebu and Remo areas southwestern Nigeria. *Journal of Medicine and Medical Sciences*. 2010 Nov 1; 1(10):485-9.
19. Mbanugo, J.I. and Agu, V.C. (2006) Prevalence of Cryptosporidium parvum Infections in Children, Aged 0-15 Years, in Anambra State, Nigeria. *Nigerian Journal of Parasitology*, 26,1-5. <http://dx.doi.org/10.4314/njpar.v26i1.37720>
20. Erin AD, Ives RL, Molloy S, Rose JB-Cryptosporidium and Giardia in surface water: A case study from Michigan USA to inform management of rural water systems. *Int J Environ Res Public Health*. 2014 Oct 11(10):10480-10503.
21. Feng Y, Xiao L. Zoonotic potential and molecular epidemiology of Giardia species and giardiasis. *Clinical Microbiology Reviews*. 2011 Jan 1; 24(1):110-40.
22. Fuchsli HP, Köttsch S, Egli T. Cryptosporidium spp. in drinking water. *Swiss medical weekly*. 2012 Oct 4; 142:w13683.
23. Garcia LS, Bruckner DA. *Diagnostic medical parasitology*. Washington, DC. 2001:131-5.
24. Garcia LS, Current WL. Cryptosporidiosis: clinical features and diagnosis. *Critical reviews in clinical laboratory sciences*. 1989 Jan 1;27(6):439-60.
25. Hamilton RJ, Facmt RJ. *Tarascon Pocket Pharmacopoeia 2014 Deluxe Lab-Coat Edition*. Jones & Bartlett Publishers; 2013 Dec 4.
26. Hotez P, Ottesen E, Fenwick A, Molyneux D. The neglected tropical diseases: the ancient afflictions of stigma and poverty and the prospects for their control and elimination. In *Hot Topics in Infection and Immunity in Children III 2006* (pp. 23-33). Springer US.
27. Hunter PR, Thompson RA. The zoonotic transmission of Giardia and Cryptosporidium. *International journal for parasitology*. 2005 Oct 31;35(11):1181-90.
28. Jeevitha D, Pradeep PS, Kanchan M-Comparative study of the prevalence of intestinal parasites in low socio-economic areas from south Chennai, India. *Journal of Parasitology Research: Vol 2014(2014): 630968*.
29. Johnson, P. D. Martin, R. Burrell, L. J. Grabsch, E. A. Kirska, S. W. O'Keeffe, J. Mayall, B. C. Edmonds, D. Barr, W. Bolger, C. Naidoo, H. & Grayson, M. L. (2005) Efficacy of an alcohol/chlorhexidine hand hygiene program in a hospital with high rates of nosocomial methicillin-resistant Staphylococcus aureus (MRSA) infection *Medical Journal of Australia* 183(10) 509-14, 2005 Nov 21.
30. Damon Callahan, (2010): unpublished thesis). Accessed in September 2015 :DOI: 10.1654/4461.1 URL: <http://www.bioone.org/doi/full/10.1654/4461.1>
31. Kaur R, Rawat D, Kakkar M, Uppal B, Sharma VK. Intestinal parasites in children with diarrhea in Delhi, India.
32. Keiser J, Utzinger J. Efficacy of current drugs against soil-transmitted helminth infections: systematic review and meta-analysis. *Jama*. 2008 Apr 23;299(16):1937-48.
33. LeChevallier MW, Norton WD, Lee RG. Occurrence of Giardia and Cryptosporidium spp. in surface water supplies. *Applied and Environmental Microbiology*. 1991 Sep 1; 57(9):2610-6.

34. MacKenzie WR, Hoxie NJ, Proctor ME, Gradus MS, Blair KA, Peterson DE, Kazmierczak JJ, Addiss DG, Fox KR, Rose JB, Davis JP. A massive outbreak in Milwaukee of *Cryptosporidium* infection transmitted through the public water supply. *New England journal of medicine*. 1994 Jul 21; 331(3):161-7.
35. McDonald S, Berzano M, Ziegler P, Murphy TM, Holden NM. Qualitative risk assessment of surface water contamination with *Cryptosporidium* sp. oocysts: A case study of three agricultural catchments. *Human and Ecological Risk Assessment: An International Journal*. 2011 Jul 1; 17(4):813-25.
36. McGeer, A. "Hand Hygiene by Habit". *Infection prevention: practical tips for physicians to improve hand hygiene*. Ontario Medical Review, November 2007, 74.
37. Mehlhorn, Heinz (2008). *Encyclopedia of parasitology* (3rd ed.). Berlin: Springer. p. 646. ISBN 9783540489948.
38. Nichols RA, Connelly L, Sullivan CB, Smith HV. Identification of *Cryptosporidium* species and genotypes in Scottish raw and drinking waters during a one-year monitoring period. *Applied and environmental microbiology*. 2010 Sep 1; 76(17):5977-86.
39. Niehaus MD, Moore SR, Patrick PD, Derr LL, Lorntz B, Lima AA, Guerrant RL. Early childhood diarrhea is associated with diminished cognitive function 4 to 7 years later in children in a northeast Brazilian shantytown. *The American journal of tropical medicine and hygiene*. 2002 May 1; 66(5):590-3.
40. Pantenburg B, Dann SM, Wang HC, Robinson P, Castellanos-Gonzalez A, Lewis DE, White AC. Intestinal immune response to human *Cryptosporidium* sp. infection. *Infection and immunity*. 2008 Jan 1;76(1):23-9.
41. Peng MM, MESHNICK SR, Cunliffe NA, Thindwa BD, Hart CA, Broadhead RL, Xiao L. Molecular epidemiology of cryptosporidiosis in children in Malawi. *Journal of Eukaryotic Microbiology*. 2003 Jul 1; 50(s1):557-9.
42. Pittet D, Hugonnet S, Harbarth S, Mourouga P, Sauvan V, Touveneau S, Perneger TV. Effectiveness of a hospital-wide programme to improve compliance with hand hygiene. *The Lancet*. 2000 Oct 14; 356(9238):1307-12.
43. Partners for Parasite Control. Meeting (3rd : 2004 : Geneva, Switzerland) & World Health Organization. Strategy Development and Monitoring for Parasitic Diseases and Vector Control Team. (2005). *Deworming for health and development : report of the third global meeting of the partners for parasite control*. Geneva: World Health Organization. <http://www.who.int/iris/handle/10665/69005>
44. Ribes JA, Seabolt JP, Overman SB. Point prevalence of *Cryptosporidium*, *Cyclospora*, and *Isospora* infections in patients being evaluated for diarrhea. *American journal of clinical pathology*. 2004 Jul 1;122(1):28-32.
45. Richardson DJ, Callahan KD, Dondji B, Tsekeng P, Richardson KE- Prevalence of waterborne protozoan parasites in two rural villages in the West province of Cameroon. *Comp Parasitol*. 78(1), 2011, pp. 180-184.
46. Stauber CE, Elliott MA, Koksal F, Ortiz GM, DiGiano FA, Sobsey MD. Characterisation of the biosand filter for *E. coli* reductions from household drinking water under controlled laboratory and field use conditions. *Water science and technology*. 2006 Aug 1; 54(3):1-7.
47. Tchuenté LT, N'goran EK. Schistosomiasis and soil-transmitted helminthiasis control in Cameroon and Côte d'Ivoire: implementing control on a limited budget. *Parasitology*. 2009 Nov 1; 136(13):1739-45.
48. WHO, *Basic Laboratory Methods in Medical Parasitology*, World Health Organization, Geneva, Switzerland, 1991.
49. World Bank. 2015. *Cameroon - Third Phase of the Community Development Program Support Project (English)*. Washington, D.C. : World Bank Group. <http://documents.worldbank.org>
50. World Health Organization. *Laboratory Basic Methods in Medical Parasitology*. A Publication of Geneva World Health Organization, (1992); pp: 11-55.
51. World Medical Association. Declaration of Helsinki. Ethical principles for medical research involving human subjects. <http://www.wma.net/e/policy/b3.htm>. 2008.