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Prevalence of *Cryptosporidium parvum* and Other Enteropathogens among Children with Diarrhoea in Benin City, Nigeria

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ABSTRACT

Fecal samples from one hundred and fifty eight (158) patients of which one hundred and thirty eight (138) were diarrheal patients and twenty are non-diarrheal persons which served as control were examined microscopically for *Cryptosporidium* oocysts in a year-long prospective study at nine (9) different hospitals in Benin City. *Cryptosporidium* oocysts were detected in 43(31.2%) of 138 diarrheal patients other pathogens included cysts of *Entamoeba histolytica* (8.7%), and *Giardia lambi*a (5.8%), ova of *Ascaris lumbricoides* (8.0%) and hookworm (2.2%), *Escherichia coli* (16.7%), and *Salmonella typhi* (2.9%). but were undetectable in 20 non-diarrheal controls between August, 2002 and October, 2003. In 19 (44.19%) of 43 positive patients, no other enteropathogens where detected. However there was no significant percentage different with oocyts isolated among the age groups (P>0.05). The percentage distribution of common pathogens in relation to the total number of isolated pathogens in the diarrheal patients indicate *Entamoeba histolytica* having 11.5%, *Giardia lambi*a 7.7%, *Cryptosporidium oo*cysts 41.3%, *Escherichia coli* 21.1% *Salmonella typhi* 3.9%, Hookworm 2.9%, and *Ascaris lumbricoides* (10.6%). Generally a higher number of cases were detected during the rainy month than the dry season. Diarrhea continued for >14days (persistent diarrhea) in 4 of the 19 cases. Almost all the patients recovered with oral rehydration. A level of personal hygiene should be maintained and this can be achieved through health education and outreach campaigns.

Keywords: Patients, Feces, Diarrheal and Non-diarrheal, *Cryptosporidium*, Enteropathogens, Oral Rehydration.

Introduction

Today, microbial health threats are once more a source of concern; these diseases are in some cases resurgent, for example tuberculosis and some completely new to human, for example cryptosporidiosis, human immunodeficiency syndrome (AIDS), and COVID 19. The reasons for emergence differ from disease to disease.

This study focuses on the disease, cryptosporidiosis in Benin City which has recently become a topic of great interest in the western world. There has been so much interest in emerging infectious diseases that a journal is now devoted exclusively to the topic; symposia have been held at various academic and research institutes and programs of studies focusing on this field have been created (CDC, 1990; CDC, 1998).

The emergence of diseases affecting human is due to many factors, depending on the particular disease. It may be due to the expansion of human habitat into the niche of a virulent organism or its vector, as in Lyme disease (Berende, 2016). It may also be a new strain of an existing microbe, as in drug-resistant forms of tuberculosis or changes in climate and/or ecology, giving rise to new infections such as the 0139 strains of Cholera (Cheesbrough, 2000). A new niche for infection may also be created by environmentally acquired or genetic immune deficiencies on the part of the host. The epidemic of Acquired Immune Deficiency Syndrome (AIDS) is one of such examples which have left humans vulnerable to many opportunistic infections. The main factor contributing to disease emergence is increased host susceptibility and infection formerly harmless or at most acute, self-limiting now pose a serious threat to life.

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Another example though less dreadful is cryptosporidiosis. This has become a cause for concern mostly due to a predisposing condition (immune suppression) on the part of its human hosts, mediated by the AIDS epidemic.

Cryptosporidium parvum is a parasitic protozoan that causes human cryptosporidiosis; this has also been observed in certain animals, including domestic livestock. In humans it causes abdominal pain, profuse diarrhea, weight loss, loss of appetite and anorexia, but in otherwise healthy individuals the infection is usually self-limiting and resolves within a few weeks (Collinet-Adler and Ward, 2010).

The infection is more serious in immune compromised patients and malnourished children, it can become chronic and is sometimes fatal (Dagan *et al.*, 1995, Guerrant, 1997). *Cryptosporidium parvum* complete its life cycle in a single host and the oocyst is highly infectious. These oocysts are usually transmitted by contaminated water, fecal transmission from infected animals, person-to-person spread or contaminated food, parasite emerging from the spores invade the epithelia cells lining the small intestine and cause the cells to form internal structure that house the parasite. The structure supplies the parasite nutrient and positions them at the interface between the digestive tract and its content (Striepen, 2013). There is no definitive cure for the disease.

Material and Methods

Area of study

The study area is Benin City, a busy cosmopolitan is the capital of Edo State, Nigeria. A total of Nine (9) hospitals and Clinics were designated for this study because of their locations and accessibility to the populace.

The Central Hospital which is a government owned hospital and the most patronized hospital and eight private hospitals namely; Akugbe Medical Center, Faith Mediplex, Bambi Hospital, Narrow Way Clinic, Mordi Clinic, St. Philomena, Gift Medical Centre and Niger clinic all located within Benin City, Edo State were the health facilities of choice for the study.

Subject

A total of one hundred and fifty eight (158) patients who visited the state hospital and eight private hospitals within the state between August, 2002 and October, 2003 were the subjects of this study. One hundred and thirty eight (138) of these were diarrheal patients aged six months to five years while the Control of the study consisted of twenty (20) aged matched Children who had no diarrhea prior to the study (Wasif *et al*. 2004). All the one hundred and fifty-eight (158) patients were screened for *Cryptosporidium* cyst and for other enteropathogens.

Sample collection

One plain clean plastic leak-proof labelled sample container was given to each patient (note sample collection was done with the assistant of the patient's mother or an adult that is with the patient), the sample was retrieved a day after the sample container was distributed.

This process was repeated on day 3, 5, 10, 15 and 20 depending on the number of days the patients were admitted in the hospital. Samples were immediately taken to Bacteriology Laboratory, Central Hospital for analysis; each sample was shared into two portions. One portion of the sample was used for bacteriological examination and the second portion for parasitological examination.

Sample Processing and Macroscopic Examination

Laboratory techniques for stool examination and special method for oocysts as described by Cheesbrough, 2000 was employed. Each stool sample was examined macroscopically for consistency, color, mucus, blood, odour and presence of adult worm such as *Ascaris lumbricoides*, hook worm or segment of tapeworm.

Microscopic Examination

Three methods were employed for each stool sample; direct wet mount microscopy, formol-ether concentration and modified Ziehl-Nelson stained method for *Cryptosporidium parvum* oocyst.

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Direct wet mount

A match stick head size of stool sample was emulsified with normal saline and iodine, cover slip was applied and slide was examined microscopically with x10 and x40 objectives. Specimens were scored as positive if intestinal parasite forms (ova or cyst) were seen.

Formol ether oocyst concentration

One (1g) gram of stool sample was properly emulsified in 4ml of 10% formol water in a test tube, additional 3ml of 10% formol water was added and sieved into a beaker. The filtrate was transferred into glass test tube into which 4ml of ether was added and shaken. The content was centrifuged at 1000 rpm for 1minute, using Pasteur pipette the entire column of fluid below the faces debris and ether was carefully removed and transferred to another test tube; 3ml of 10% formol water was added to the tube and centrifuge at 3000rpm for 10minutes after centrifugation the supernatant was discarded and the sediment was used to prepare a smear for modified Ziehi-Nelson stain (Cheesbrough, 2000).

Modified Ziehi-Nelson method for oocyst

Smear was made on a clean slide free of grease from the sediment obtained from the ether oocyst concentration, air dried and fixed with methanol for 3 minutes. The slide was then flooded with unheated carbol fuchsin for 15 minutes, rinsed and counter stained with 0.5% methylene blue for 30 seconds, the prepared slide was rinsed, allowed to air dry and examined microscopically for oocyst using x10 and x100 objective.

Bacteriological Examination

A loopful of stool sample was inoculated on MacConkey agar plate and Selenite F bottle and incubated aerobically at 37^{o}C overnight. Following incubation, a loopful of selenite F culture was subculture on Deoxychocolate Citrate agar (DCA) plate; incubated aerobically at 37^{o}C for 24hours. The MacConkey plate and DCA were examined; suspected colonies on the culture media were identified using API 20 E system.

Principle of API 20 system

The API 20 E consists of a plastic strip with 20 micro tubes containing dehydrated biochemical substrates. These strips were incubated with bacterial suspension that reconstitutes the media. During incubation metabolism produces color changes that are either spontaneous or revealed by addition of reagents. The reactions were read according to the reading table and identification obtained referring to analytical profile index.

Use of API 20 E

Preparation of the strip, inoculums and inoculation were done based on the manufacture's instruction. Reading of strip and identification were made by referring to reading and identification provided in the test kit.

Results

The results obtained from the one hundred and fifty-eight (158) patients screened for *Cryptosporidium* cyst revealed that no *Cryptosporidium* oocyst was detected in the twenty non-diarrheal that served as control. On the other hand, out of the 138 samples obtained from the diarrheal patients, ninety (90) (65.2%) patients had common enteropathogens including common intestinal pathogens while forth-eight (48) (34.8%) were without pathogen.

Table 1 indicates the frequency of fecal *Cryptosporidium oo*cyst in diarrheal patients and non-diarrheal controls. Forty three (31.2%) diarrheal patients had *Cryptosporidium oo*cysts detected with an isolation rate of 22.2% in children less-than one year old, 39.1% in ages 1-3years and 26.3% in ages greater than 3 to 5years. There was however no significant percentage difference among the age groups ($P > 0.05$).

Table 2 shows the duration of diarrheal and *Cryptosporidium oo*cysts excretion in severe and persistent cases with 26.3%, 75% and 100% of oocysts excretion in persistent cases of diarrheal while 25%and 73.7% in severe cases. Contingence analysis of Table 2 indicated significant relationship between oocyst excretions with persistence of diarrhea (X^2, $P < 0.001$).

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Table 1: Frequency of fecal Cryptosporidium oocyst in diarrheal patients and non – diarrheal controls

Age group (yrs.)	Diarrheal Patients		Non-diarrheal Patients	
	No examined	Cases with oocyst (%)	No examined	Cases with oocyst (%)
< 1	36	8(22.2)	8	0(0)
1 – 3	64	25(39.1)	9	0(0)
> 3 – 5	38	10(26.3)	3	0(0)
Total	138	43(31.2)	20	0(0)

Table 2: Persistent excretion of Cryptosporidium oocysts in diarrheal patients

Duration of diarrheal (Days)	Cases examined	Severe cases (%)	Persistent cases (%)
≤ 7	38	28 (73.7)	10 (26.3)
8 – 14	4	1(25)	3 (75)
15 – 21	-	-	-
22 – 28	-	-	-
29 – 35	-	-	-
36 – 42	1	-	1 (100)

Percentage distribution of common pathogens in the diarrheal patients as shown in Figure 1 indicate *Entamoeba histolytica* having 8.7%, *Giardia lambi*a 5.8%, *Cryptosporidium oo*cysts 31.2%, *Escherichia coli* 16.7% *Salmonella typhi* 2.9%, Hookworm 2.2%, and *Ascaris lumbricoides* (8.0%).

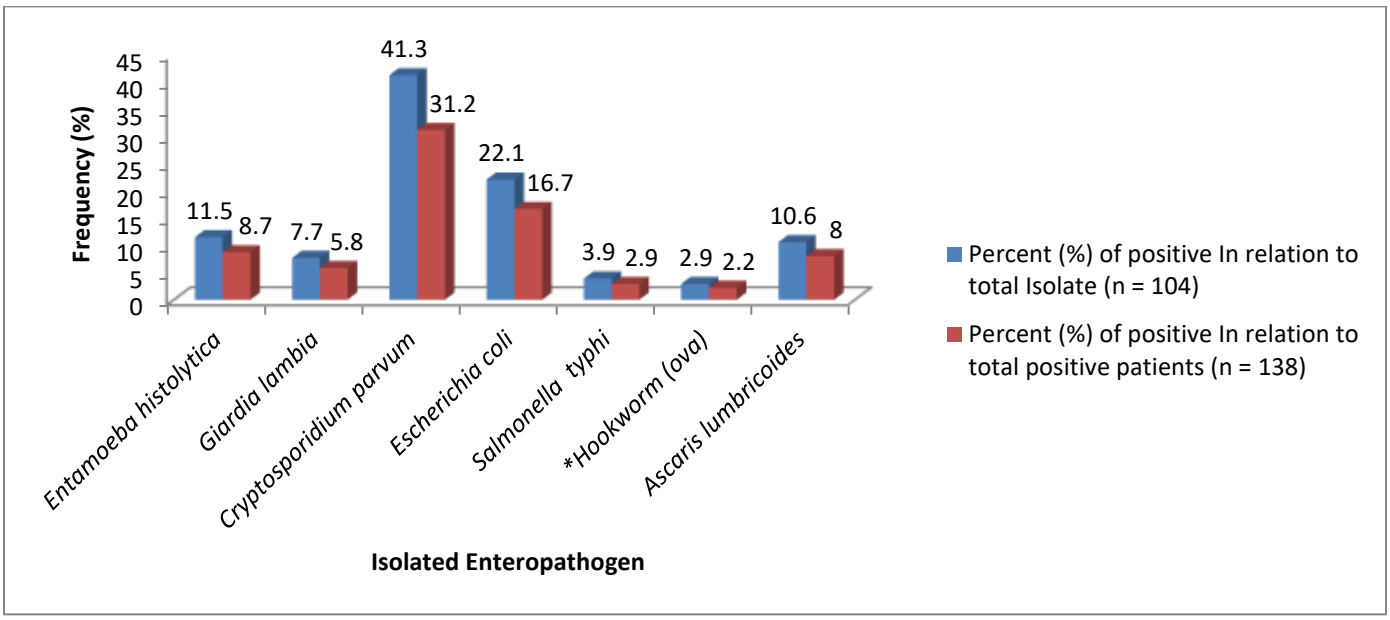

Figure 1: Enteropathogens isolated from diarrheal patients

The percentage distribution of pathogens in relation to the total number of isolated pathogens from diarrheal patients as shown in Figure 1 indicate *Entamoeba histolytica* having 11.5%, *Giardia lambi*a 7.7%, *Cryptosporidium oo*cysts 41.3%, *Escherichia coli* 21.1% *Salmonella typhi* 3.9%, Hookworm 2.9%, and *Ascaris lumbricoides* (10.6%). *Cryptosporidium oo*cysts was identified as a single pathogen in 19 patients while *Cryptosporidium* species were associated in five (5) cases with *Entamoeba*

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histolytica; in three (3) cases with *Giardia lambia*; *Ascaris lumbricoides* in six (6) cases; *Escherichia coli* in eight (8) cases and hook worm in two (2) cases. Symptoms with *Cryptosporidium* infection included diarrhea (100%), vomiting (74%), dehydration (73%), anorexia (47%), running nose (20%), fever (28%) and abdominal pain (22%). Intravenous rehydration was required only in five (5) patients. One patient died during the course of treatment while other patients recovered after correcting dehydration orally.

Slight indication of seasonal occurrence was observed with higher numbers appearing during the rainy months as presented in Figure 2.

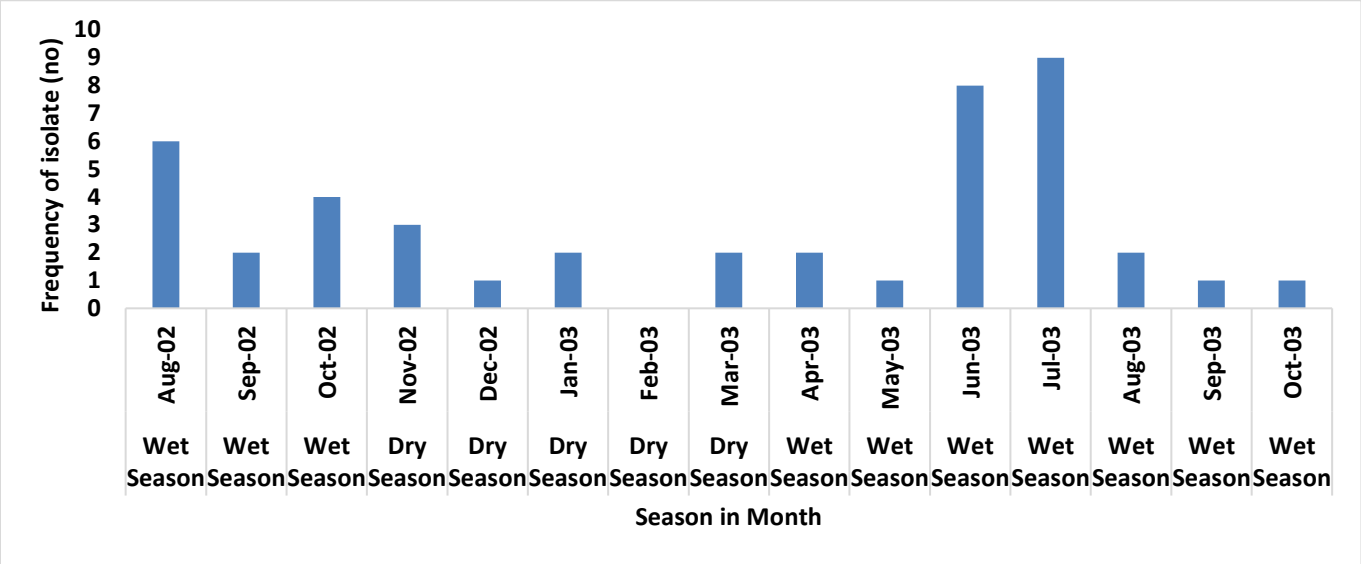

Figure 2: Seasonal occurrence of *Cryptosporidium* in diarrheal patients

Discussion

This present study reveals the prevalence of *Cryptosporidium* among children with diarrhea in Benin City between August 2002 and October 2003. As at the time of reporting this study, it was the first study that was ever carried out on *Cryptosporidium* in Benin City, Nigeria. This study shows that *Cryptosporidium* is associated with diarrheal illness among children in Benin City. One hundred and thirty eight (138) patients were tested, 31.2% were positive; 19 (44.2%) had *Cryptosporidium* as a single enteropathogen. The result is in line with Wang *et al.* (2015) finding, where 60% tested positive in New York City, otherwise higher when compared with other developing countries. Shahina *et al.* (2010) noted 9% in North West of Pakistan, Mahbubur *et al.* (1990) recorded 4.8% in Bangladesh while Dabas *et al.* (2017) obtained 1.7- 35% in Indian. There are possibilities that the result could be higher since all cases of diarrhea were not reported. In the study, API 20 E was used in order to rule out any enteric bacteria; 2.9% of *S. typhi* and 17.7% *Escherichia coli* were found in association with *Cryptosporidium*. This is low compare to the percentage isolation of *Cryptosporidium*. The result also revealed that *G. lamblia* and *Entamoeba histolytica* were identified but also at lower percentage 8.9% and 13.3 % respectively. No *Shigella* was isolated. Assessment for viral agent was not performed. Slight occurrence in seasonality (Fig. 2) was observed with the higher number appearing during the raining months of the year especially in the months of June – November than in other months. This may be attributed to unhygienic nature of the patients who has formed habit of picking from the ground into their mouth. The seasonality was also noted from other studies but mostly during the wet month, Mare *et al.* (1990) in Philippines. In Bangladesh, Mahbubur *et al.* (1990) noted high cases of detection during hot and humid month (April – July), also observed is Cryptosporidiosis in calves and their handlers; the infection rate was found higher (14.2%) during the month of May – November.

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Cryptosporidium though less dreadful is known to cause persistent diarrhea. This is said to be a cause for concern mostly due to predisposing condition (immunocompromised) on the part of the patients, which may be mediated by AIDS epidemic and also in malnourished children with diarrhea (Guerrant, 1997).

In this study a year old child who had persistent diarrhea died within 14 days, this may be due to the predisposing condition has the child was found seropositive (HIV +). Observed also is the persistent diarrhea significant number of children (Table 2) Analysis of result revealed that *Cryptosporidium* oocyst excretion in diarrheal patients significantly influenced the duration of diarrhea ($P < 0.001$).

Unlike Cryptosporidiosis in AIDS patients, the persistent diarrhea in these patients was self-limited, albeit prolonged. It is interesting to note that in this study persistent diarrhea cases excreted oocysts for longer periods than severe diarrhea case. Thus a persistent diarrheal case could serve as a source of infection in the community.

Conclusion

The study demonstrates that *Cryptosporidium* is relatively common cause of diarrhea in children particularly in the rainy months of the year in Benin City. It is therefore recommended that for every stool sample sent to the laboratory, a proper investigation including diagnosis for *Cryptosporidium* oocyst should be made in both government and private laboratory.

To the clinicians who may be faced with severe diarrhea pediatric cases and with evidence of *Cryptosporidium* as the causative agent, it is recommended that intensive support care be given to the patient since there is no curative measure. Level of personal hygiene should be maintained and this can be achieved through health education.

Competing Interests

The author declares no conflict of interests.

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