Assessment of Maternal Cryptosporidium Infection and Risk Factors among Antenatal Attendees in Makurdi, Benue State, Nigeria

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ABSTRACT

In order to determine prevalence, risk factors and ascertain association of diarrhoea symptoms of maternal Cryptosporidium infection in Makurdi, 510 feacal samples were randomly collected from volunteered consented antenatal attendees in Makurdi Township. The stool samples were concentrated using formol-ether oocyst concentration technique, stained with modified Ziehl Neelson cold stain and oocysts identified by microscopy. Human Immunodeficiency Virus (HIV) serological status was determined using Determine (Inverness, Japan) and confirmed with Uni-Gold HIV1/2 (Trinity Biotech, USA). Chi square ($\chi 2$) in an SPSS (version 20.0) software was used to compare categorical variables and significant association was determined at 95% level of probability. Cryptosporidium oocyst was identified in 98(19.2%) of the faecal samples examined. HIV positive attendees were significantly more infected with Cryptosporidium (84.4% vs 9.9%, $P<0.001$) than their HIV negative counterparts. We found a strong association between stool consistencies ($P < 0.001$), duration of diarrhoea ($P < 0.001$), possession of domestic animals ($P < 0.001$) and Cryptosporidium infection rate. There was also a significant difference ($P < 0.001$) between the rate of infection and sources of drinking water. However, infection could not be associated with age ($P=0.121$), type of toilet system in use ($P=0.793$) and literacy ($P=0.665$). Pregnant women in this study suffer a high burden of Cryptosporidium infection which could complicate HIV status of those immunocompromised culminating into diarrhoea-related mortality. Routinely all antenatal attendees with diarrhoea should be tested for Cryptosporidium in this setting.

Key words: Cryptosporidium, Risk factors, pregnant women, Diarrhoea, Makurdi

INTRODUCTION

Infections with *Cryptosporidium* parasites are described to be an important underlying cause of diarrhoea in developing countries, infecting a vast range of other organisms and humans especially children and immunocompromised persons (Brett *et al*., 2003; Kwaga *et al*., 1988, Xiao and Feng, 2008).

It has been evident that as few as 132 oocysts of *Cryptosporidium parvum (C. parvum)* are capable of initiating infection in healthy persons (Dupon *et al*., 1995). Nearly 20 Cryptosporidium species have been detected in humans, among which *Cryptosporidium hominis* (*C. hominis*) and *C. parvum* are the most reported species (Khan *et al*., 2019). The spread of these parasites occur through feacal-oral route and also by consumption of contaminated water or food and/or zoonotic or anthropogenic transmission (Yoder *et al*., 2010). *C. parvum* oocysts are able to live in nature for 18 months, provided the environment is cool and damp/wet, and are reportedly, quite common in lakes and other water sources that have at one time been contaminated with sewage or manure (Safe Drinking Water Foundation, 2017).

Makurdi town is facing problem of acute water supply that can be traced to the poor and/or lack of adequate operation and maintenance practices of improved water sources (Aper and Agbehi, 2011). This lack of proper water sources has led to the complete dependence by residents on water sources from unprotected shallow wells and the river used for various purposes that may not be totally hygienic (Utsev and Aho, 2012). As a result, a great percentage of the population is at high risk of water borne diseases such as diarrhoea, typhoid, and cholera to mention but a few (Ocheri *et al*., 2012).

Infection with Cryptosporidium is reported to begin in the first few months of life but clinical episodes of Cryptosporidium-associated diarrhea illness peaked at 6 11 months (Sow *et al*., 2016). In a Tanzanian prospective cohort study, maternal *Cryptosporidium* infection and maternal hand washing prior to infant feeding was associated with increased likelihood of infant *Cryptosporidium* infection (Petersen *et al*., 2014).

Notwithstanding, diagnosis of maternal feacal samples for *Cryptosporidium* parasites is not a common practice in Makurdi but is suspected of contributing to childhood diarrhoea related mortality. A number of works have been carried out on *Cryptosporidium* in Nigeria (Inyang-Etoh *et al*., 2007; Molly *et al*., 2011; Atu *et al*., 2016; Anejo-Okopi *et al*., 2016) but none targeted pregnant women who could serve as a source of infection to neonates and infant. Hence, this study was aimed at determining the prevalence, associated risk factors and to ascertain the association of Cryptosporidium infection with diarrhea symptoms among antenatal attendees in Makurdi.

MATERIALS AND METHODS

Study Area

Makurdi the capital of Benue State, Nigeria with an estimated population of 500,797 (Federal Government Population Gazette, 2009), lies between latitude $7^{O}30$, $7^{o}43$'N and longitude 8^{o} 30, $8^{o}35$'E with an altitude of about 90metres above sea level. The city is located on the banks of the Benue River, a tributary of River Niger, and is divided into two; North Bank and South bank. Due to the general low relief, sizeable portions of Makurdi are water logged and flooded during heavy rainstorms. This is reflected in the general rise in the level of groundwater in wells during wet season (Chia *et al*., 2014). Monthly temperature is usually between $27^{o}C$ and $28^{o}C$ and may rise to about 30 $40^{o}C$ on the maximum. The area receives between 900 1000mm of rain on an annual basis with dry season beginning from late

October to March. The rainy season usually spans from April to early October and is the major window for agricultural activities.

Ethical Considerations and Approval of the study

The ethical committee of Benue State Ministry of Health and Human services approved the study design and written consent procedures. Permission was also obtained from head of the two healthcare centres in Makurdi Township. Verbal consent was sought and obtained from the antenatal attendees of Bishop Murray Medical Centre and Police Clinic all in Makurdi.

Data Collection and Sample Analysis

Questionnaire was use to obtain data of each antenatal attendee on demography, clinical information and some possible risk factors that might be related to *Cryptosporidiosis. An infected individual was described as a pregnant woman with Cryptosporidium oocysts in the stool at that time.*

Sample size was determined using the formula (Naing *et al*., 2006).

$$n = \frac{z^2 P(1 - p)}{d^2}$$

Where, n = sample size; z = statistic for a level of confidence, in this case the level of confidence was 95% (1.96)

p = expected prevalence which in this case was 20% (0.2) and

d = precision at 5% (0.05)

After counselling by professional medical health practitioners a total of 510 feacal samples were collected from volunteered consented antenatal attendees who were available at the 2 Health Care centres (Bishop Murray Medical Centre, 328 and Police Clinic, 182) in Makurdi Township

Stool samples were collected in clean universal containers and processed within 30 minutes. Samples that were not to be processed immediately were preserved in 10% formalin.

Sample of venous blood (3mL) was aseptically collected from each of the 510 participants by venepuncture into a vacutainer using a standard procedure. The blood was allowed to clot and centrifuged at 1000 g for10 minutes under ambient temperature to separate the cells from serum. Serum was extracted using Pasteur pipettes and was stored in cryogenic vials at −17°C if serum was not to be used immediately.

Formol Ether Oocyst Concentration Technique

The stool samples were concentrated using formol-ether oocyst concentration technique as described by Cheesbrough (2005). One millilitre of a well-mixed stool sample was introduced into a tube containing 4mL of the 10% formalin (Formol water) and mixed by shaking. Additional 3mL of formol water was added, shake and the suspension sieved using a coffee strainer into a centrifuge tube made of copolymer. Four millilitres of diethylether was added and stoppered. Vigorous mixing of the tube was made, the stopper removed and the suspension centrifuged at 400g for 1 minute. The entire column of formol water below the ether and faecal debris was transferred into another tube using Pasteur pipette, followed by addition of formol water to make up to 10mL. The suspension was centrifuged at 1000g for 10 minutes, the supernatant was discarded and the deposit was used in making smear for staining.

Modified Ziehl Neelsen Staining

Air-dried smeared glass slide was fixed with absolute methanol for 3 5 minutes. Carbol funchsin solution was added to the slide covering the whole smear for 15 minutes. The slide was washed with tap water using a wash bottle. Decolourization was done with acid alcohol (1% Hydrochloric acid Ethanol) and counter stain with malachite green (Chesbrough, 2005). The microscopic examination of the slide for Cryptosporidium oocysts was done using 100x (oil immersion) objective. Cryptosporidium oocyst appeared as bright rose-pink on a pale green background.

Screening of Serum Samples for Human Immunodeficiency Virus (HIV)

HIV sero-status was determined using Determine rapid test strip (Inverness, Japan) and confirmed with Uni-Gold HIV1/2 (Trinity Biotech, USA).

All test were carried out in Microbiology Laboratory, Department of Biological Sciences, Benue State University Makurdi, Nigeria.

Data Analysis

The data generated was entered into an SPSS (version 20.0) software. The association between categorical variables and the prevalence of Cryptosporidium infection was estimated using chi square (χ^2) test with a significant association determined at 95% level of probability ($P \leq 0.05$).

RESULTS

Of the 510 antenatal attendees that participated in the study, Cryptosporidium oocysts was detected in 98 (19.2%). HIV positive attendees had significantly higher Cryptosporidium than their the HIV negative counterpart (84.4% vs 9.9%; χ^2 = 200.164, df = 1, P < 0.001), However, cryptosporidium infection rate was not different among the age groups (P = 0.121), Occupation (P = 0.102) and literacy levels of the sampled attendees (P = 0.665) (Table 1)

Unformed stool samples were more liable (χ^2 = 134.109, df = 2, P < 0.001) to oocyst detection (58.8%) than formed (9.1%) and semi-formed (7.2%) samples (Table 2)

Similarly, attendees that reported having diarrhoea within the last 6 months were more infected (54.4%) than those without diarrhoea (6.4%; χ^2 =147.994, df = 1, P< 0.001), and the infection rate was proportional to the duration of the diarrhoea

Table 1: Distribution of Cryptosporidium infection according to Demographic characteristics and HIV Status of Ante natal Attendees in Makurdi, Nigeria

Demographic Characteristic	Number Examined	Number Positive (%)	P-value
Age			
16 - 25	200	46(23.0)	0.121
26 - 35	234	36(15.4)	
36 - 45	76	16(21.1)	
Occupation			
Civil servant	82	8(9.8)	0.102
Business Woman	190	36(18.9)	
Farmers	92	24(26.1)	
Student	66	14(21.2)	
Others	80	16(20.0)	
Literacy			
Non- formal	24	6(25.0)	0.665
Primary	64	14(21.9)	
Secondary	210	42(20.0)	
Tertiary	212	36(17.0)	
HIV Status			
Positive	64	54 (84.4)	0.000**
Negative	446	44 (9.9)	

** Highly significant

Table 2: Questionnaire Responses to History of Risk factors and Cryptosporidium infection status for each response group of Antenatal Attendees in Makurdi Metropolis

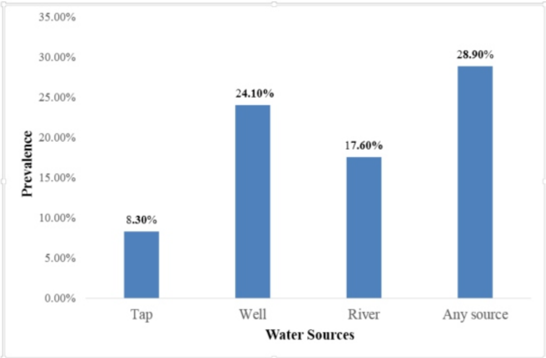
Parameter	Response Sought	Number of Responders	Number infected	P-value
Access to treated water	Yes	60	12 (20.0)	0.870
	No	450	86 (19.1)	
Contact with Animals	Yes	172	54 (31.4)	0.000**
	No	338	44 (13.0)	
Swimming	Yes	84	20 (23.8)	0.408
	No	426	78 (18.3)	
Diarrhoea in the last 6 months	Yes	136	74 (54.4)	0.000**
	No	374	24 (6.4)	
Duration of Diarrhoea	1 – 2 days	60	12 (20.0)	0.001**
	3 – 5 days	46	34 (73.9)	
	7 and above	28	26 (92.9)	

** Highly significan

Table 3: *Cryptosporidium* infection in relation to Sampled Stool consistency of Antenatal Attendees in some selected Hospitals in Makurdi metropolis.

Stool Consistency	Number examined	Number infected (%)
Formed	110	10 (9.1)
Semi formed	276	20 (7.2)
Unformed	124	68 (54.8)
Total	510	98 (19.2)

χ^2 = 134.104, df = 2, p < 0.001



X^2 21.128, df = 3, P = 0.000

Figure 1: Distribution of Cryptosporidium Infection in Relation Drinking Water Sources Used by Antenatal Attendees of Some Selected Hospitals in Makurdi Metropolis.

Table 4: Distribution of *Cryptosporidium* infection with respect to toilet systems available for use by antenatal attendees of some selected hospitals in Makurdi metropolis

Toilet system	Number examined	Number infected (%)
Pit latrine	92	20 (21.7)
Water cistern	376	70 (18.6)
Open defecation	42	8 (19.0)
Total	510	98 (19.2)

$\chi^2 = 0.465$, df = 2, p = 0.793

been highest among those with diarrhoea lasting for more than 7 days (92.9%; χ^2 =52.119, df = 2, P < 0.001)

The study also recorded higher cryptosporidium infection rate among attendees who had contact with domestic animals (31.4%) than those who do not (13.0%; χ^2 = 24.801, df = 1, P < 0.001) (Table 3)

Questionnaire responses to sources of drinking water utilized by the antenatal attendees and cryptosporidium infection for this response group shows higher infection among those that used any available source (28.9%), followed by those that had hand dug well as their source of water (24.1%) and the infection rate was significantly higher (χ^2 = 21.128, df = 3, P < 0.001) those that had access to municipal tap water supply (Figure 1).

DISCUSSION

Prevalence of maternal *Cryptosporidium* infection (19.2%) reported in this study is moderately high compared to 9.4% reported (Atu *et al*., 2016) among children in orphanages in Benue State. Higher rate recorded in this study may not be unconnected to the fact that the findings were not *Cryptosporidium* species specific contrary to the report of Atu *et al*. (2016) that focused on *Cryptosporidium parvum. However, prevalence of 19.2% recorded in this study is comparable to 19.4% reported (Molloy et al., 2011) among children in Ile-Ife town of Osun State, Nigeria.*

High prevalence of Maternal *Cryptosporidium* infection reported here could translate to early infant infection especially among babies whose mothers practice force feeding using bare hands that may not be well washed (Petersen *et al*., 2014; Ayinmode and Obebe, 2017). Cryptosporidiosis in early childhood has been associated with subsequent impairment in growth, physical fitness and intellectual capacity (Mead, 2014).

Our study established a strong association between Cryptosporidium infection and diarrhea (P<0.001) in the study population similar to the findings of Anejo-Okopi *et al*. (2016) in Jos, Nigeria. Though, cryptosporidiosis is reported to be self-limiting in immunocompetent individuals (Mead, 2014; Saulawa *et al*., 2016), significantly higher infection associated with longer duration of diarrhea in this report could be due to suppressed immunity (84.4% prevalence among HIV positive attendees) of a good number of attendees. This is collaborated by prevalence of *Cryptosporidium parvum* of 97.7% reported among HIV subjects in some selected health care facilities in Osun State (Adesiji *et al*., 2007).

Though, highly active antiretroviral therapy (HAART) is reported to improve immunity and reduces the occurrence of enteroparasitic infections (Akinbo *et al*., 2010), the study did not sought the impact of HAART on prevalence of cryptosporidium infection. However, high Cryptosporidium prevalence reported among HIV positive subjects in this study may not be unconnected with lack of awareness and routine based testing (Khan *et al*., 2019).

With significant heavy burden of Cryptosporidium associated with different drinking water sources, there is even greater risk of complicated diarrhea disease among HIV positive individuals in this study. This is more so when Cryptosporidium infested feaces are washed into shallow wells and streams serving as sources of domestic water supply. Rapid inactivation of 90 % oocyst have been experimentally documented in tap water using solar radiation but with an increased dissolved organic content in the environment, cryptosporidium subsists due to decrease in oocyst solar inactivation (King *et al*., 2008). This becomes more apparent when the temperature of the surface water and shallow wells is lower than 60°C (Fayer, 1994) as was the case of the studied location where temperature was between 27°C and 28°C during the period of the study (November, 2017 - January, 2018).

Significantly higher Cryptosporidium infection observed in this study among attendees that had contact with animals further explains high infection in those that had wells and river as their main source of water. The high prevalence of the *C. parvum* reported in cattle, sheep and goats (Akinkuotu and Fagbemi, 2014) and the high number of oocysts that is reportedly shed by infected animals make cattle, sheep and goats important sources of environmental contamination with *Cryptosporidium* oocysts that are able to infect humans (WHO, 2009).

This may be made worse with the insurgent of herd of cattle, sheep and goats increasing open grazing witness by the general Benue State communities. Pregnant women in this study suffer a moderate burden of *Cryptosporidium* infection which could complicate HIV status of those immunocompromised culminating into diarrhoea-related mortality. Extensive hand washing be employed by all individuals. Direct contact with stool from animals or humans should be avoided. Drinking of raw (untreated) water should be avoided and ranching of animals should be encouraged to reduced contamination of water bodies. Routinely all attendees that presented with diarrhoea should be tested for *Cryptosporidium* and treated.

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