Cryptosporidium parvum infection and nutritional status of children under-five years in some selected hospitals in Kaduna State, Nigeria.

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Abstract- Background of the study. Diarrhoea is one of the leading causes of death among children under five globally. In a study carried out to determine the prevalence rates of some parasites implicated in diarrhoea among children aged 0-5 years and their nutritional status in Kaduna State, Nigeria, stool samples were collected from 368 children in selected hospitals representing the three senatorial districts of the State. The result obtained showed prevalence of 19.8% and 22.8%, for *Cryptosporidium parvum*, using both microscopy and Enzyme Linked Immunosorbent Assay. The result of this study showed that the percentage of the study population which were underweight, stunting and wasting which were 17.4%, 27.4% and 11.4% respectively. The Z-score values obtained for underweight, stunting and wasting were < -2, < -2 and < -2 standard deviation(SD), all within the 'low' range. There was no statistical significant association between *Cryptosporidium parvum* and gender (χ^2=0.098, df=1, OR=1.083 and p-value=0.754). The infection with *C. parvum* had no association with age, source of drinking water, water treatment and type of toilet. Although the association between the gender, age and the aforementioned risk factors, the high prevalence of *C. parvum* observed in this study indicates that this parasite is prevalent within the study area and as such the consumption of Well protected and treated drinking water should be considered a priority for reducing the existing high prevalence rates of *Cryptosporidium parvum* within the study population and area.

Index Terms- Diarrhoea, *Cryptosporidium parvum*, Underweight, Stunting, Wasting, Kaduna State.

I. Introduction

Diarrhoea is defined as the occurrence of loose or watery stools at least three times per day, or more frequently than normal for an individual (UNICEF, 2009). Diarrhoeal disease is the second leading cause of death in children under five years old. It is both preventable and treatable (WHO, 2013a). Undernourished children are at higher risk of suffering more severe, prolonged and often more frequent episodes of diarrhoea. Repeated bouts of diarrhoea can also undermine children's nutritional status because of their decreased food intake and reduced nutrient absorption, combined with their increased nutritional requirements during repeated episodes. Diarrhoea often leads to stunting in children due to its association with poor nutrient absorption and appetite loss (UNICEF, 2013). *Cryptosporidium parvum* is an important specie implicated in diarrhoea (Mims *et al*., 1999). It is increasingly recognized as the parasitic pathogen responsible for diarrhoea in developed and developing areas among immunocompetent and immunocompromised persons (Wolfson *et al*., 1985). *Cryptosporidium parvum* is an intracellular protozoan parasite that is associated with gastrointestinal diseases in all classes of vertebrates including mammals, reptiles, birds, and fish. The organisms infect and reproduce in the epithelial cells of the digestive or respiratory tracts. Infection is predominantly associated with diarrhoea and biliary tract disease (Chen *et al*., 2002). The sequence of events in the pathogenesis of cryptosporidiosis is thus: after invasion of the erythrocytes, the epithelial cells release cytokines. These cytokines activate phagocytes and recruit new leukocytes which in turn, release soluble factors – resulting in intestinal secretion of chloride and water – and inhibit absorption. Enterocyte damage maybe a direct consequence of parasite invasion, multiplication, and extrusion. Regardless of the specific mechanism, marked distortion of the villus architecture is accompanied by nutrient malabsorption and osmotic diarrhoea. *Cryptosporidium* species have been identified in every continent except Antarctica (O' Connor *et al*., 2011).

II. Materials and Methods

Study Area

Kaduna State is located on the southern end of the high plains of northern Nigeria. With respect to the estimate conducted in 2006, it has a population of 6,066,562 and 23 Local Government Areas. The bedrock geology is predominantly metamorphic rocks of the Nigerian Basement complex consisting of biotic gneisses and older granites. Kaduna State experiences a typical continental climate with distinct seasonal regimes, oscillating between cool to hot dry and humid to wet. The soil and vegetation are typical reddish brown to reddish yellow tropical ferruginous savannah grassland with scattered trees and woody shrubs. Agriculture is the main stay of the economy of the State, with about 80% of the people actively engaged in farming. Cash and food crops are cultivated and the produce include yam, cotton maize, beans, guinea corn, millet, ginger, rice and cassava. Kaduna State is the leading producer of cotton in Nigeria. Animal rearing and poultry farming is also a major occupation in Kaduna State.

Study Design and Population

The study was hospital and clinic based. The study population were infants and young children aged 0-5 years presenting with or without diarrhoea at or hospitalized in the health care facilities in the state.

Ethical Clearance

Ethical approval was obtained from the State Ministry of Health and Ethics Committee of hospitals before the study commenced. (MOH/ADM/744/VOL.1)

Inclusion and Exclusion Criteria

The inclusion criteria included: children aged 0-5 years of both sexes, diarrhoeic and non – diarrhoiec , and informed consent. Children who have been on antimicrobial agents were excluded from the study. The exclusion criteria included: age above 5 years of both sexes, and lack of consent.

A diarrhoeic case in the study was defined as a child passing loose, watery or a bloody stool three or more times in a 24 hour period as reported by parents.

Sample Collection

At the respective health facility, arrangements were made with resident doctors, matrons or laboratory technicians whereby the mother or caregiver of any child who satisfies the study inclusion criteria was asked to provide the child's stool and blood specimen after consultation. Prior to collection of specimen, the mother or caregiver who consented to inclusion of his/her child in the study was interviewed using structured questionnaire designed to obtain demographic and clinical information about the child and his/her illness. Samples were collected when available. Where possible, stool samples were collected on the spot from the child using potty lined with cellophane for each use. Otherwise, the mother or caregiver was asked to bring it the following day. All specimens were submitted to the health facility laboratory and refrigerated where possible or placed in an ice box until they were transported to the laboratory in the Department of Microbiology, Ahmadu Bello University Zaria, Kaduna State, Nigeria for analysis.

Sample processing by Formol-Ether concentration Technique

This sedimentation technique is rapid and has the advantage of removing lipid and colloidal material to yield clear sediment. In addition, the presence of formalin preserves eggs, larvae, and cysts so that the material can be examined hours or even days later.

The 1 gram or about a pea size of the stool sample was emulsified in a 15ml conical test-tube containing 10ml of 10% formalin, and centrifuged at 3000 rpm for 5 minutes. After which the supernatant was decanted. To the sediment, 10ml of 10% formalin, and 4ml of diethyl ether were added, centrifuged at 3000 rpm for 5minutes. The plug of debris which was formed between the layers of the two liquids i.e. diethyl ether, was rimmed off using an applicator stick, and then decanted, leaving only the sediment in a small volume of formalin that drained back from the sides of the tube. With a Pasteur pipette, some of the sediment was fetched and used to make a smear on a glass slide and subjected to Modified Ziehl Neelsen's staining procedure (WHO, 2003). The slides were stained with carbol-fuchsin for 5 minutes and then rinsed off with water. After which the slides were stained with acid-alcohol for1 minute and rinsed off with water. The slides were counter-stained with methylene blue for 2 minutes and rinsed off with water. The slides were then placed in a rack and allowed to drain and dry. Finally, the stained slides were microscopically examined using 100X magnification. Oocysts stain pink to red or deep purple against a blue background.

III. RESULTS

Malnutrition indices among *Cryptosporidium parvum* infected and non-infected children is shown in table1. Body mass index (kg/m^2) is defined as weight in kilograms divided by the square height in meters. Undernutrition was indicated by W eight for Height –WAZ and the Z –Score was <-2 =17.4% (64/368) while stunting (Height for Age-HAZ) Z –Score was <-2 = 27.4% (101/368). The wasting (Weight for Age Z scores was <-2 = 11.4% (42/368). The prevalence of *Cryptosporidium parvum* among the study population in relation to gender, age, and some risk factors such as source of drinking water, method of water treatment practiced and type of toilet used is shown in Tables 2 and 3. Out of 368 samples, 73 samples were positive for *C.parvum* by microscopy.

Table 1: Malnutrition indices among *Cryptosporidium parvum* infected and non-infected children

Nutritional Index	Gender	Infected children		Non-infected children		Total	
		N	%	N	%	N	%
WHZ	Male	13	27.7	34	19.9	47	21.6
	Female	2	7.7	15	12.1	17	11.3
	Total	15	20.5	49	16.6	64	17.4
HAZ	Male	16	34.0	50	29.2	66	30.3
	Female	10	38.5	25	20.2	35	23.3
	Total	26	35.6	75	25.4	101	27.4
WAZ	Male	10	21.3	21	12.3	31	14.2
	Female	4	15.4	7	5.6	11	7.3

Total	14	19.2	28	9.5	42	11.4

HAZ=Height-for-Age, WAZ =Weight-for-Age (WAZ), and WHZ=weight-for-height.

Table2: Prevalence of *Cryptosporidium parvum* infection to gender and age

	Microscopy		ELISA		
Variables	**No Positive**	**No Negative**	**No Positive**	**No Negative**	**Total**
Gender					
Male	47(21.6)	171(78.4)	51(23.4)	167(76.6)	218
Female	26(17.3)	124(82.7)	33(22.0)	117(78.0)	150
P-value	**0.318**		**0.754**		
Age (Months)					
0-12	35(24.1)	110(75.9)	38(26.2)	107(73.8)	145
13-24	11(12.9)	74(87.1)	13(15.3)	72(84.7)	85
25-36	11(22.4)	38(77.6)	12(24.5)	37(75.5)	49
37-48	11(23.9)	35(76.1)	12(26.1)	34(73.9)	46
49-60	5(11.6)	38(88.4)	9(20.9)	34(79.1)	43
P-value	**0.150**		**0.390**		
Total	**73(19.8)**	**295(80.2)**	**84(22.8)**	**284(77.2)**	**368**

Crypto**M**: (**Gender**: χ^2=0.998, **df**=1, **OR**= 1.311 and **p-value**=0.318)(**Age**: χ^2=6.544, **df**=5 and **p-value**=0.257)
Crypto **E:** (**Gender**: χ^2=0.098, **df**=1, **OR**=1.083 and **p-value**=0.754)(**Age**: χ^2=6.544, **df**=5 and **p-value**=0.257)

Table3: Prevalence of *Cryptosporidium parvum* in relation to some risk factors

Factors	**Microscopy**		**ELISA**		
	No Positive	**No Negative**	**No Positive**	**No Negative**	**Total**
Source of Drinking Water					
Pipe-borne	19(14.7)	110(5.3)	30(23.3)	99(76.7)	129
Well	37(24.8)	112(75.2)	33(22.1)	116(77.9)	149
Borehole	7(12.7)	48(87.3)	11(20.0)	44(80.0)	55
River/Stream	1(25.0)	3(75.0)	1(25.0)	3(75.0)	4
Others	9(29.0)	22(71.0)	9(29.0)	22(71.0)	31
P-value	**0.095**		**0.911**		
Water Treatment					
Boiling	5(11.9)	37(88.1)	8(19.0)	34(81.0)	42
None	58(21.6)	210(78.4)	60(22.4)	208(77.6)	268
Filtering	8(19.5)	33(80.5)	12(29.3)	29(70.7)	41
Others	2(11.8)	**15(88.2)**	4(23.5)	13(76.5)	17
P-value	**0.406**		**0.720**		
Toilet Type					
Pit latrine	44(20.9)	167(79.1)	44(20.9)	167(79.1)	211
Open field	3(21.4)	11(78.6)	3(21.4)	11(78.6)	14
Water closet	19(15.8)	101(84.2)	31(25.8)	89(74.2)	120
Others	7(30.4)	16(69.6)	6(26.1)	17(73.9)	23
P-value	**0.393**		**0.744**		
Total	**73(19.8)**	**295(80.2)**	**84(22.8)**	**284(77.2)**	**368**

P-value = level of significance

IV. Discussion

From this study, out of 368 stool samples collected from children presenting with diarrhoea in some selected hospitals in Kaduna State, 73 and 84 samples were found to be positive for *Cryptosporidium parvum* by microscopy and ELISA respectively. The result obtained showed prevalence rates of 19.8% and 22.8% for *C. parvum* and this prevalence is rates are lower than the 27.6% reported in a similar study conducted in Lagos by Oyibo *et al*. (2008). This study has shown higher prevalence rate than the 8.1% which was reported in Ethiopia (Tigabu *et al*., 2010). It is also lower than the prevalence rate of 5.6% which was reported in Nepal (Shariff *et al*., 2002). These

differences observed may be due to the experimental design. In relation to gender, the prevalence rate for *C. parvum* was higher among male children than females. This may be because male children are more active than females and as such engage in more outdoor games than their female counterpart and in the course of this, may pick up the parasite and become infected. Children within the age range of 6-11 months recorded the highest prevalence for *C. parvum* in the present study. This result does not agree with the report by Yilgwan and Okolo, (2012) in Jos where the highest prevalence for *C. parvum* was recorded among children within the 49-60 months of age. The reason for this difference may be due to the feeding and weaning practices employed by the mothers or care givers of these children (Yilgwan and Okolo, 2012) and the level of hygiene maintained.

Analysis of the result by source of drinking water showed that there was no significant association between *C. parvum* and the source of drinking water, yet higher prevalence rates were recorded among those who happen to have rivers/streams and sources other than pipe-borne, well or borehole as their sources of water. This report agrees with the report by Boadi and Kuitunen, (2005), who claimed that low incidence of diarrhoea is associated with water sources such as pipe-borne, borehole but increases with lack of access to safe water Shier *et al*. (1996).
Although the prevalence of *C. parvum* was high among those who claimed not to apply any form of treatment to their water before drinking, there was no significant association between the prevalence of *C. parvum* and type of water treatment.

Similarly, there was no significant association between the infection with *C. parvum* and type of toilet used by the subjects within the study population but it was discovered that the lowest prevalence rate for *C. parvum* was recorded among those who use water closet as a toilet facility. This report agrees with the report of Boadi and Kuitunen, (2005), who stated that the incidence of diarrhoea has a high association with the lack of access to good toilet facilities and also that water closets and pit latrines are associated with substantial reductions in diarrhoea.

The nutritional status of the children within the population was assessed using the indices WHZ as Weight – for – Height, HAZ as Height – for – Age and WAZ as Weight – for – Age and by the standards of Food and Agriculture Organization (FAO) of the United Nations to determine the percentage of the study population which were underweight, stunting and wasting which were 17.4%, 27.4% and 11.4% respectively. The Z-score values obtained for underweight, stunting and wasting were < -2, < -2 and < -2 standard deviation (SD), all within the 'low' range. This implied that the children within the study population are moderately underweight, stunted or wasted (FAO, 1996).

V. Conclusion

This study was able to show the prevalence rate for Cryptosporidium parvum among children aged 0-5 years attending some selected hospitals in Kaduna State was 19.8% and 22.8% for both microscopy and ELISA. The Zscore value which is expressed as the nutritional status of the population is <-2 Standard Deviation (SD) which by Food and Agriculture Organization (FAO) of the United Nations standard falls within a low range thereby making the study population moderately undernourished.

VI. Recommendations

Well protected and treated drinking water should be considered a priority for reducing the existing high prevalence rates of *Cryptosporidium parvum* within the study population and area.

Proper waste and sewage disposal practices should be maintained so as to reduce the chances of infection.

The step – wise procedure involved in preparing homemade Oral Rehydration Therapy/ Salt Sugar Solution (ORT/SSS) should not only be taught in hospitals and clinics during antenatal visits but also through the media, so that women who do not give birth to their children in the hospitals can also learn about and administer ORT/SSS to children if they come down with diarrhea.

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