Prevalence of Rotavirus and *Cryptosporidium parvum*Co-infection among Children with Acute Gastroenteritis in Zaria, Kaduna State, Nigeria

[1*]Gambo A., [2]Inabo,H.I. and[2]Aminu, M.
[1]Department of Microbiology, Faculty of Life and Chemical Sciences Usmanu Danfodiyo University, Sokoto, Nigeria
[2]Department of Microbiology, Faculty of Life SciencesAhmadu Bello University, Zaria, Nigeria
Corresponding author: contactauwal@gmail.com (08038407057)

Abstract: This research was conducted to determine the prevalence of rotavirus and *Cryptosporidium parvum* co-infection among children with acute gastroenteritis in Zaria, Nigeria. A total of 340 diarrhoeic stool samples and 32 age matched control of children aged 0-60 months were screened for the presence of *C. Parvum* and rotavirus antigen using ELISA. Out of 340 diarrhoeic samples screened, 11(3.0%) samples were positive for both *C. Parvum* and rotavirus antigens (co-infection) and 329 (96.76%) were negative for the co-infection. Rotavirus was exclusively positive in 73(20%) samples while *C. Parvum* was exclusively positive ein 28(8%) samples. Total of 260(70%) samples were negative for the two pathogens. Of (3%) co-infection detected, 8(4%) were among 199 males and 3(1.7%) were among 173 females children studied. Age of the patients (p=0.028), source of drinking water (p=0.036), not exclusive breast feeding (p=0.014), weaning at early infancy (p=0.010) were some of the risk factors associated with the co-infection.

Key words: Prevalence, Rotavirus, *Cryptosporidium parvum,* Children, Gastroenteritis, Zaria

INTRODUCTION

Gastroenteritis is a serious health problem largely because the aetiology and pathogenesis of persistent diarrhoea are usually multifactorial and sometimes cannot be identified (Ogbu *et al*., 2008). In general, the same pathogens are responsible for gastroenteritis worldwide, with only variations in the frequency of occurrence of each pathogen in different localities (Carlos and Saniel, 1990). Gastroenteritis can be caused by a number of agents, including viruses, bacteria, parasites and toxins (Carroll and Reimer, 2000; Sherif *et al*., 2003). Some cases have one single defined aetiology, others do not have any defined cause, and a substantial number (one third) are caused by multiple pathogens (Lindsay *et al*., 2011). Many rotavirus infections occur as mixed infections with other microorganisms, including viruses, bacteria, and protozoa (Argüelles *et al.,* 2000; Ajjampur *et al.,* 2007; Lindsay *et al*., 2011; Ira *et al*., 2019).Rotavirus was found to be the dominant etiology of severe diarrhea even in vaccinated children(Ira *et al*., 2019).It has shown that infection with *C. parvum* in children from developing countriespredisposes to substantially increased diarrhoeal illnesses (Guerrant *et al.,* 2002).

MATERIALS AND METHODS

Sample preparation for Antigens Detection by ELISA

For the detection of rotavirus antigens, faecal suspension was prepared in 1:5 dilutions by mixing 1gm of stool into 4ml of diluted wash buffer. It was then mixed well and the mixture was left for heavy particles to settle. While for the detection of *C. parvum* antigens the faecal suspension was prepared in 1:4 dilutions by mixing 0.1gm of stool into 0.3ml of dilution buffer (Manufacturer's Instruction). Rotavirus and *C. parvum* antigens screening were conducted in all the stool specimens by using the commercially available enzyme Linked Immuno-Sorbent Assay (ELISA) (Diagnostic Automation Inc) (23961 Craftsman Road, Suite E/F, Calabasas, CA 91302 USA Cat. No. 8306-3) according to the manufacturer's instructions.

Procedures for Antigens Detection

i. Rotavirus Detection

The kits containing ninety-six (96) micro-wells were placed on the laboratory experiment bench. Each micro-well contains anti-rotavirus polyclonal antibodies. Well number 1 was left as blank. Approximately 100µl of the negative and positive control were added to the second and third well respectively. About 100µl of the faecal suspension was added to the remaining micro-wells and the plate incubated at room temperature for thirty minutes (30 minutes) as described by the manufacturer. After the incubation period, the plate was then washed using diluted wash buffer and two drops of reagent 1 (a blue solution containing anti-rotavirus monoclonal antibodies) was then added to each well (excluding the blank) incubated for 5 minutes and washed with wash buffer thereafter. Two drops of reagent 2 (a red solution containing anti-mouse antibodies conjugated to horse radish peroxidase) was also added to each well (excluding the blank) incubated for 5 minutes and washed with wash buffer thereafter. Two drops of chromogen was then added to each well and incubated for 5 minutes.Some wells turned to blue at this point. After the incubation period, 2 drops of stopping solution (sulphuric acid) were then added to each well. Distinct yellow colour was produced in the blue wells and the absorbance was read spectrophoto-metrically at an absorbance of 450nm.

ii. *Cryptosporidium parvum* Detection

The first well of 96 micro-wells was left blank. About 100µl of negative and positive control were added to the second and third wells respectively. Approximately 50µl of dilution buffer was added to each micro-well excluding blank, positive and negative control. About 50µl of faecal suspension was added to all wells excluding blank and the controls. This was then incubated for an hour at room temperature and washed thoroughly with wash buffer thereafter. Two drops of enzyme conjugate were then added to each well and washed thoroughly after thirty (30) minutes incubation period. Furthermore, two drops of chromogen were added to each well and incubated for 10 minutes at room temperature. This was followed by the addition of 2 drops of stopping solution (sulphuric acid) to each well. Distinct yellow colour was produced in some wells and the absorbance was read spectrophoto-metrically at an absorbance of 450nm.

RESULTS

The results of the screening for rotavirus and *C. parvum* antigens confirmed the prevalence of the co-infection of the two diarrhoeic pathogens among the study population.Out of the 372 stool samples screened, 11(3.0%) samples were positive for both *C. Parvum* and rotavirus antigens (co-infection) and 329 (96.76%) negative for the co-infection. Rotavirus was exclusively positive in 73(20%) samples while *C. parvum*was exclusively positive in 28(8%) samples (Table 1). Total of 260(70%) samples were negative for the two pathogens. Of 11 (3%) co-infection detected, 8(4%) were among 199 males and 3(1.7%) were among 173 females children studied (Table 2). However, the association between the co-infection and the sex of the children was statistically insignificant (χ^2 = 1.685, df = 1, p=0.194).

Further analysis of the data showed significant association between the co-infection and the age of the children (χ^2 = 18.657, df = 9, p=0.028). Highest prevalence of the co-infection occurred among 19-24 moths age group (13.6%: 6/44) followed by 13-18 months age group (5.7%: 2/35). Less number of cases was recorded among other age brackets (Table 2). No co-infection was recorded after 2 years of age.

Table 1: Prevalence of Rotavirus and *C. parvum* infection among children with acute gastroenteritis in Zaria, Nigeria

No Examined	No Negative (%)	No Positive with Rotavirus (%)	No Positive with *C. parvum* (%)	No with Co-infection (%)
340	329 (96.76%)	73 (21.5%)	28 (8.2%)	11 (3.23%)

Table 2: Prevalence of rotavirus and *C. parvum* co-infection in relation to sex among children with acute gastroenteritis in Zaria, Nigeria.

Population	No. Examined	No. Positive	%Prevalence	p value
Sex				
Male	181	8	4.4	0.194
Female	159	3	1.9	
Age (months)				
0-6	78	2	2.7	
7-12	111	1	1.0	
13-18	35	2	5.7	
19-24	44	6	13.6	0.028
25-30	28	0	0	
31-36	18	0	0	
37-42	3	0	0	
43-48	8	0	0	
49-54	6	0	0	
55-60	9	0	0	

The result however, showed there was statistically significant association between co-infection and breast feeding practices (χ^2 = 17.608, df = 1, p=0.030) (Table 3). Higher prevalence of the co-infection were noted among those not breast fed (7.5%) than among breast fed children (1%) (Table 3). Furthermore, statistically significant association was noted between the co-infection and exclusive breast feeding (χ^2 = 8.463, df = 1, p=0.014). The prevalence of the co-infection was higher among children who were on mix feeding (6%) than among children who were exclusively breast fed (1.9%) (Table 3). Similarly, there was statistically significant association between *C. Parvum* and rotavirus co-infection and the age of weaning (χ^2 = 1.608, df = 1p=0.010) (Table 3). Prevalence of co-infection was highest among 0-6 months age group (50%) (Table 3) followed by 19-24 months age group (11.6%).

Table 3: Prevalence of rotavirus and *C. parvum* co-infection in relation to breast feeding practices among children with acute gastroenteritis in Zaria, Nigeria.

Mode of feeding	No. Examined	No. Positive	% prevalence	p-value
Breast milk	220	2	1.0	
Breast milk substitute	120	9	7.5	0.030
Exclusive breast milk				
Exclusive	52	1	1.9	
Mix feeding	168	10	6.0	0.04
Age of weaning(months)				
0-6	2	1	50	
7-12	36	0	0.0	
13-18	31	3	9.6	0.00
19-24	43	5	11.6	
25-30	8	0	0	

The results obtained showed insignificant association between the presence of household pet and the co-infection of the two pathogens (χ^2 = 0.348, df = 4, p=0.986). Nevertheless, highest prevalence of the co-infection occurred among children from household with only dogs (4.6%) (Table 4). This was followed by the prevalence of the co-infection among children from household with other animals (3.6%). Least prevalence was recorded from household with only cats (2.7%). No co-infection was recorded from houses with both cats and dogs (0%) (Table 4). The association between the prevalence of the co-infection and the source of drinking water was statistically significant (χ^2 = 6.685, df = 4, p=0.036). All the incidences of the co-infection occurred among children from households dependent on the well water (5.1%) (Table 4).

There was statistically significant association between rotavirus and *C. parvum* co-infection and the type of toilets in the study population (χ^2 = 12.907, df = 2, p=0.023). All the incidences of the co-infection occurred among children from household using pit latrine (3.5%). No Co-infection was recorded among other type of toilets (Table 4).

Table 4: Prevalence of rotavirus and *C. parvum* co-infection in relation to the presence of household pets, source of water and type of toilets among children with acute gastroenteritis in Zaria, Nigeria.

Variable	No. Examined	No. Positive	% Prevalence	p-value
Presence of pets in the households				
Dogs only	22	1	4.6	
Other animals	111	4	3.6	
Cats only	112	3	2.7	0.986
Without animal	92	3	3	
Both cats and dogs	3	0	0	
Source of water				
Well	214	11	5.1	
Stream	44	0	0	0.036
Pipe borne	78	0	0	
Bore-hole	3	0	0	
Type of toilet				
Pit latrine	316	11	3.5	
Water Closet	20	0	0	
In the field	4	0	0	0.023

Key:*Other Animals = Animals apart from cats and dogs, such as sheep, goats, cattle etc. *Without animals = not pets and other animals

DISCUSSION

The result of this study confirmed the presence of rotavirus and *C. Parvum* co-infection in the study population. This finding is corroborated by the report of Japhet *et al.* (2019) of the incidence of rotavirus co-infection with other viruses in South Western Nigeria. Grimprel *et al.* (2008) noted in a report of rotavirus infection that many rotavirus infections occur as mixed infection with other microorganisms, including viruses, bacteria, and protozoa. This incidence is also in agreement with the findings of Luyandu *et al.* (2020) who reported the occurrence of rotavirus-*Cryptosporidium* co-infection among South African children. This result is also supported by the assertion of Valentiner-Branth *et al.* (2003) who in a cohort study of Guinean children indicted *C. Parvum* as one of the pathogenic parasites that cause diarrhoea among children in association with rotavirus. Presence of co-infection in some of the samples investigated in this study is in line with the occurrence of co-infection reported from other parts of the world such as Nepal (Sherif *et al*., 2003) and India (Lindsay *et al.,* 2011).

No significant association was observed between the co-infection and the sex of the children.Many studies have reported the insignificant association between rotavirus and the sex of the patients (Pennap and Umoh, 2010;Junaid *et al*., 2011;Sherchand *et al*., 2011:Gambo*et al.,* 2016;Aliyu *et al*., 2017; Oyinloye *et al*., 2017; Ojobor *et al*., 2020) and between *C. parvum* infection and sex of the study population (Egberongbe *et al*., 2010 and Gambo *et al*., 2014 and Anejo-Okopi *et al*., 2016; Shinkafi and Muhammad).

The prevalence of the co-infection in relation to the age of the children was statistically significant. This is consisted with the reports of many researchers who reported significant association between rotavirus infection and age (Aminu *et al*., 2008; Pennap and Umoh, 2010;Junaid *et al*., 2011;Sherchand *et al*., 2011; Gambo *et al.,* 2016; Oyinloye *et al*., 2017; Luyandu *et al.,*2020; Ojobor *et al*., 2020) and*C. parvum* infection and age (Nwabuisi, 2001;Egberongbe *et al.* 2010; Odeniran and Ademola, 2019).

All the co-infection occurred among children within 0-24 month age bracket. Many researchers have reported the occurrence of highest prevalence of rotavirus infection within this age bracket (Pennap and Umoh, 2010; Junaid *et al*., 2011 and Ojobor *et al*., 2020) and highest prevalence of *C. parvum* in this age group (Nwabuisi, 2001; Odeniran and Ademola, 2019). This tends to suggest that children within 0-24 month age are more prone to *C. Parvum* and rotavirus co-infection.

The association of rotavirus and *C. parvum* co-infection and breast feeding practices was statistically significant.Highest prevalence of the co-infection occurred among not breast feeding children. This is in agreement with the reports of Aminu *et al*., 2008,Pennap and Umoh (2010) and Gambo *et al.* (2016) who speculated that breast feeding confer some protection against rotavirus infection.This finding also agreed with the assertion by Mor and STzipori, (2008) that breast-feeding is assumed to provide some protection against *C. parvum* infection through conferment of immunoglobin. Breast feeding from this finding therefore, confer some protection against rotavirus and *C. parvum* co-infection as most of the co-infection occurred among not breast feeding children.

There was not statistically significant association between rotavirus and *C. parvum* co-infection and presence of household pets in this study. This result is consistent with the reports of non-zoonotic association of *C. parvum* infection in humans by other researchers such as Gambo *et al.* (2014) in Zaria. It is also in agreement with the reports of Chacín-Bonilla *et al.* (2008) in Venezuela, Mor and Tzipori, (2008) in Sub-Saharan Africa and Ayinmode *et al.* (2012) in Oyo Nigeria. This result also agreed with the report of non-significant association between rotavirus infection and house hold pets by Parashar *et al.* (2006a).

Robertson *et al*. (2020) reported in a review study the anthropologic transmission of cryptosporidiosis. It can be deduced from this finding that the co-infection of rotavirus and *C. parvum* are transmitted to humans in ways other than animal-human route. This finding is contrary to the observed significant association between *C. parvum* infection and the presence of house hold pets noted by some researchers who attributed cryptosporidiosis to zoonotic transmission (Keusch *et al*., 1995; Meinhardt *et al*., 1996). The reason for this contradiction is not understood.

This study revealed that the prevalence of rotavirus and *C. parvum* co-infection was significantly associated with the source of drinking water. This finding is consistent with the report of significant association between *C. parvum* and source of drinking water from South Western Nigeria by Egberongbe *et al.* (2010) and Anejo-Okopi *et al.* (2016) from Jos North Central Nigeria. The report is also consisted with reports from other parts of the world such as Jordan (Nimri and Batchoun, 1994) South Africa, (Omoruyi *et al.,* 2011) Ireland (Garvey and McKeown, 2009) and USA (Keusch *et al.,* 1995). This significant association between the co-infection and the source of drinking water contradicted some reports that dissociated rotavirus infection and source of drinking water (Parashar *et al*., 2006b; Aminu *et al*., 2008; Pennap and Umoh, 2010; Gambo *et al*., 2016).

This finding is corroborated by the report of Anejo-Okopi *et al.* (2016) of significant association between *Cryptosporidium* infection and source of water. It has been documented that *Cryptosporidium* oocysts can survivein water and hence be transmitted,thereby causing diarrhoea in the vulnerable consumer (Nimri and Batchoun, 1994; Keusch *et al*., 1995; Fayer *et al.,* 2000). This could serve as the reason for the contradiction in the significant association between the co-infection and the source of drinking water in this study. All the co-infection occurred in houses dependent on wells as source of water. It was observed in the course of this investigation that most of the wells in the study area are shallow hence can be easily contaminated with human and animal excreta. It can be concluded from this finding, that source of drinking water served as means through which *Cryptosporidium* oocyst is transmitted in this study area which play an important role in the co-infection.

The association between the prevalence of rotavirus and *C. parvum* co-infection and the type of toilets was statistically significant. This is consisted with the study of Gambo *et al*. (2016) and Oyinloye*et al.* (2017) who reported the association between rotavirus infections and the type of toilet used in the house hold.Similar observation was made by Parasha *et al.* (2006). This finding also supported the report of Egberongbe *et al.* (2010) from south western Nigeria, who associated type of toilet with *C. parvum* infection. This is corroborated by the recommendation of CDC (2011) that, good hygiene (hand washing after use of toilets) and cleanliness are important but are not enough to control the spread of the disease. This means the diseasecanspread in the absence of or improper hand washing after defecating and/or before meals through which the virus and the oocyst can be transmitted.

CONCLUSION

There exists arotavirus and *C. parvum* co-infection in Zaria. Source of drinking water, type of toilets and breast feeding practices play important role in the rotavirus and *C. parvum* co-infection among the study population. it is recommended that proper citing of toilets with considerable distance from wells and other source of house hold water should be strictly adhere to. Measures should be taken to include testing for rotavirus and *Cryptosporidium* in the diagnosis of children with diarrhoea.

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