



Bayero Journal of Pure and Applied Sciences, 9(1): 39 - 47

Received: March, 2016

Accepted: May, 2016

ISSN 2006 – 6996

DETECTION OF ROTAVIRUS INFECTION IN CHILDREN WITH GASTROENTERITIS ATTENDING THREE SELECTED HOSPITALS IN KANO METROPOLIS, NIGERIA

*¹Aminu, A. I., ²Muhammad, A. and ³Mohammad, Y.

¹Department of Microbiology, Faculty of Science, Bayero University, Kano Nigeria.

²Medical Department, Laboratory Unit, Sheik Muhammad Jidda General Hospital, Kano.

³Department of Microbiology and Parasitology, Bayero University, Kano Nigeria

*Correspondence author: email: aishatuaminuibrahim@gmail.com, 08054503326.

ABSTRACT

The study aimed at detecting the incidence of rotavirus infection among children with gastroenteritis in Kano Metropolis. It was a descriptive cross-sectional study and a total of 200 stool samples were randomly collected and assayed for the presence of rotavirus antigens using Enzyme Linked Immunosorbent Assay and confirmed using Real Time Polymerase Chain Reaction. The study revealed that 21/200 (10.5%) stool samples were positive for rotavirus antigen and more males 13/21 (6.5%) were positive than females 8/21 (4%) ($p > 0.05$). The highest and lowest incidence rates in rotavirus infection of 42.8% (9/21) and 4.8% (1/21) were found among children aged 7–12 month and 31–36; 43–48 month ($p > 0.05$) respectively. Most of the positive samples (95%) were from those who presented with diarrhea, vomiting and fever ($p < 0.05$). The study further revealed that 20/21 (95%) of the positive children were from married couples and the incidence rate was found to be independent of the educational level of the parent ($p > 0.05$). Among the risk factors considered, source of drinking water (tap water) and playing with toys appeared to be the most predisposing factors as 16/21 (76%) and 19/21 (90.5%) of the children were found to be infected ($p > 0.05$). Finally, the lowest rate of rotavirus infections was found in only 1/21 (0.5%) among the exclusively breastfed children compared to 14/21 (7%) of those on mixed feeding ($p > 0.05$). The study recommends detection of rotavirus infection to be part of routine laboratory tests in our hospitals and advocates the concept of exclusive breastfeeding to curtail rate of infection among infants.

Keywords: Rotavirus, Incidence rate, Risk factors, Children, Hospitals, Kano State.

INTRODUCTION

Rotavirus has long been acknowledged to be a major etiological agent of gastroenteritis and responsible for a large proportion of morbidity and mortality associated with diarrheal illnesses (Parashar *et al.*, 2003). According to Tate *et al.* (2012), rotavirus causes 37% of deaths attributable to diarrhoea and 5% of all deaths in children younger than five. They further revealed that each year rotavirus causes millions of cases of diarrhoea in developing countries, almost 2 million resulting in hospitalization and an estimated 453, 000 resulting in the death of a child younger than five years. In Sub-Saharan Africa and Asia, according to Kotloff *et al.*, (2013), rotavirus is one of the four most common causes of moderate to severe diarrhea among children <5 years of age. Diarrhea is one of the documented '10 killer conditions' among Ugandan children with an estimated 10,637 deaths due to rotavirus occurring annually in children < 5 years of age (World Health Organization [WHO], 2008). In Nigeria, a high incidence of childhood diarrhea is estimated to account for over 160, 000 of all deaths in children less than 5 years of age annually and of this number, approximately 20% are associated with rotavirus infection (Parashar *et al.*, 2003).

Rotaviruses belong to the Reoviridae family. There are five species of rotavirus referred to as A, B, C, D and E. Humans are primarily infected by species A, B and C, but most commonly by species A and all the five species cause disease in other such as primates, cows, horses, pigs and sheep (Kirkwood 2010; Centers for Disease Control and prevention (CDC, 2009).

Rotavirus A, which accounts for more than 90% of rotavirus gastroenteritis in humans (Leung *et al.*, 2005), is endemic worldwide. Simpson *et al.* (2007), Parashar *et al.* (2006) and Rheingan *et al.* (2006) further stated that boys are twice as likely as girls to be admitted to hospital.

Transmission of rotavirus is primarily via fecal-to-oral spread, both through close person-to-person contact, contact with contaminated environmental surfaces and respiratory droplets (CDC, 2007; Glass *et al.*, 2006; Dennehy *et al.*, 1998). The virus is environmentally hardy can survive on hands for at least four hours and remains viable on surfaces or fomites (such as toys hard surfaces) for days (Dennehy 2000; Butz *et al.*, 1993; Ansari *et al.*, 1988). Both asymptomatic and symptomatic health care workers have been linked to the spread of the virus in some outbreaks.

Fever, diarrhea and vomiting are the most common symptoms, which can occur either alone or in combination (Staat *et al.*, 2002). Clinical presentation can vary by age group and infants may be asymptomatic or only have mild symptoms with a lower likelihood of diarrhea and vomiting compared with other children (Chang *et al.*, 2003; Bass *et al.*, 2007). Gastrointestinal symptoms typically resolve within 3 to 7 days (CDC 2006) and immunity develops with each infection, so subsequent infections are less severe; adults are rarely affected (Grimwood and Lambert 2009).

The major role of rotavirus in causing diarrhea is not widely recognized within the public health community and particularly in developing countries as observed by Rodrigo *et al.* (2010) and Simpson *et al.* (2007). Despite the fact that several studies including those by Tate *et al.* (2012), Parasher *et al.* (2007) and Simpson *et al.* (2007) revealed that almost every child has been infected with rotavirus by age five and infection by the virus was identified as the leading single cause of severe diarrhoea among infants and children, and most importantly it causes 37% of deaths attributable to diarrhoea and 5% of all deaths. Thus, the need to document the existence of the infection or disease particularly in our community cannot be overemphasized especially bearing the fact that rotavirus is not routinely diagnosed in most Nigerian health facilities including hospitals in Kano. The study aimed at detecting the incidence of rotavirus infection among children less than 5 years of age in Kano State. It also described the role played by some of the demographic characteristics and identified risk factors associated with the occurrence of rotavirus infection.

MATERIALS AND METHODS

The study was a descriptive cross – sectional study conducted at three selected hospitals located in Kano metropolis (Hasiya Bayero Pediatric Hospital, Murtala Muhammad Specialist Hospital-MMSH and Infectious Diseases Hospitals-IDH). Hasiya Bayero Pediatric Hospital is the major hospital in the state where children receive medical attention; MMSH is the second largest hospital that receives patients from all over the state and IDH was established to handle cases of infectious diseases.

An ethical approval was obtained from the Kano State Hospitals Management Board. With their consent, parents who agreed to allow their children to participate in the study were asked to fill the questionnaire that assisted in identifying some of the demographic characteristics and possible risk factors associated with rotavirus infection.

All children aged 0 – 5 years who presented with signs or symptoms of gastroenteritis that came to the hospitals and were seen by the physicians/health officers from January 2014 to January 2015 were included in the study upon the consent of their parents. Children with dysentery, diarrhoea for more than 14 days, or diarrhoea developing after hospitalization due to any other cause were excluded. Children who were on immunosuppressive drugs or documented significant back-ground disease such as

immuno-deficient syndromes were also excluded from the study population.

A total of 200 stool samples were collected randomly from the selected children based on the 13.8% incidence rate of rotavirus infection reported by Junaid *et al.* (2011) in a study conducted at Jos using standard epidemiological formula described by Sarmukaddam and Gard (2006). The samples were collected using sterile, wide mouth universal containers as described by Junaid *et al.* (2011), which were given to the parents and were asked to cover and submit them immediately to the laboratories of those hospitals. The samples were then labeled and stored at -20°C until assay.

The samples were screened for the presence of rotavirus antigen according to DRG Rotavirus Ag ELISA kit (2014) and positive samples were confirmed with RT – PCR (Liferiver, 2012).

Detection of the rotavirus infection using ELISA

Detection of the rotavirus infection according to DRG Rotavirus Ag ELISA kit (2013) is a one-step enzyme immunoassay based on reaction of horseradish peroxidase (HRP) labeled polyclonal anti-Rotavirus-antibodies to the group specific VP-6 antigen, the major protein of group A Rotaviruses.

A 1:6 dilution of the sample was prepared and centrifuged for 1 minute. Then, 75 μl of horseradish peroxidase (HRP) (labeled polyclonal anti-Rotavirus-antibodies conjugate) was dispensed into each of the wells with wells 1 and 2 serving as positive control and negative control respectively. Then 50 μl of each of the diluted samples was dispensed into the appropriate test wells accordingly, mixed and incubated at room temperature for 60 minutes. The contents were then decanted and each well was washed and 75 μl of substrate solution (3,3',5,5'-Tetramethylbenzidine –TMB) was then dispensed into each of the wells, mixed and incubated at room temperature for 10 minutes without exposure to sunlight. Horseradish peroxidase (HRP) converted the subsequently added colorless substrate solution of 3,3',5,5'-Tetramethylbenzidine (TMB) within a 10 min reaction time into a blue product and the reaction was terminated by adding 75 μl of stop solution (sulphuric acid) into the wells that turned the solution from blue to yellow. The absorbance of each well was read at a wavelength of 620 nm (optical density –O.D. of the solution is directly proportional to the specifically bound amount of Rotavirus antigen) and results were interpreted as described in the user manual by DRG (2013) as follows:

Cut-off value determination = O.D of negative control + 0.2; O.D of negative control was found to be 0.02, hence, Cut-off value = 0.02 + 0.2 = 0.22

Samples with O.D \geq 0.22 were considered positive and samples with O.D below 0.22 were considered negative

Confirmation of positive samples according to RT – PCR

Confirmation of positive samples was based on the principle of real time detection based on fluorogenic 5' nuclease assay according to RT – PCR (Liferiver, 2012). The procedure was carried out according to manufacturer's instructions.

Bajopas Volume 9 Number 1 June, 2016

All the frozen stool samples were allowed to thaw and 1: 10 dilution of each of the stool samples was made using normal saline, the samples were then centrifuged for 20 minutes at 4000g, and the supernatants were collected into labeled containers. Viral RNA extraction was carried out as described by Qiamp viral RNA mini kit (2011) in a class II biosafety cabinet and stored at -60°C at PCR laboratory, Aminu Kano Teaching Hospital, Kano, Nigeria.

For preparation of mastermix, 23µl each of RT PCR enzyme mix and internal control (ic) was dispensed into a reagent container containing 414µl of super mix. This was used for 21 samples and 2 controls. Then exactly 20µl of master mix was dispensed into each of the 23 Real time PCR reaction plate wells. Then 5µl of positive control was dispensed in well 1 of the reaction plate and 5µl of negative control was dispensed in to well 2 of the PCR reaction plate. Finally, 5µl of each of the extracted RNA samples was dispensed into the respective wells corresponding to the individual label on the samples containers and the micro plate was then covered immediately and loaded into the PCR instrument for amplification. Thermal conditions for the amplification were set as follows: 45°C for 10 min, 1 cycle; 95°C for 15 min, 1 cycle; 95°C for 15 sec, 60°C for 1 min, 40 cycles. Results were read after 1 hour 37 minutes and interpreted as described by Liferiver (2014) where extracted RNA samples with cut off value ≤ 38 in FAM channel were considered positive.

Statistical analysis

Date generated from the study was analyzed using SPSS version 22 Software and Chi-square test was used to determine significant association where P-value of 0.05 or less was considered significant.

RESULTS

The results of this study show that out of the 200 screened stool samples, 21(10.5%) were positive for rotavirus using ELISA, and confirmed using RT – PCR method. The highest and lowest incidence rates in rotavirus infection of 42.8% (9/21) and 4.8% (1/21) were found among children in the age group of 7 – 12

months and 31 – 36; 43 – 48 months ($p > 0.05$) respectively (Table 1), with more males 13(6.5%) infected than females 8(4%) ($p > 0.05$) (Table 2).

The study revealed that diarrhoea was found to be significantly associated with rotavirus infection ($p < 0.05$) as most of the children (95.2%) had diarrhea in addition to other symptoms (Table 3).

With regards to the demographic characteristics of parents of the studied children, the study revealed that 20/21 (95.2%) of the positive children were from the married couples. Although the rate of rotavirus infection among the positive children was also found to be significantly associated with the educational level of the parent yet 10/21 (47.6%) of the positive children were from parents/guardian who had secondary education and only 2/21 (9.5%) where from parents/guardian who had tertiary education ($p > 0.05$), (Tables 4 and 5).

The possible risk factors associated with the rate of rotavirus infection of the children in this study were presented in table 6-12. Tables 6 and 7 revealed that the highest rate of infection of 8% (16/21) and 9% (18/21) was identified among children who had tap water as their source of drinking water and whose toilets were located far away from their source of water ($p > 0.05$). The rate, however, did not differ significantly among children that played with toys ($p > 0.05$) and among those that played with other children ($p > 0.05$) (Tables 8 and 9)

Table 10 revealed that 57.1% (12/21) of the positive samples were found among children whose hands were washed before meal ($p > 0.05$) and the highest rate of infection of 66.7% (14/21) was found in children that consumed food that did necessarily require cooking ($p > 0.05$) (Table 11).

Finally, the rate of rotavirus infection in relation to type of feeding was presented in Table 12, and the lowest rate of 0.5% was found in two children with one on exclusive breastfeeding and the other on bottle feeding while the highest rate of rotavirus infections of 66.7% was found among 14 children who were on mixed feeding ($p > 0.05$).

Table 1: Rotavirus A infection in relation to age distribution of the studied children

Age(Months)	Rotavirus Infection Status		Total (%)
	Positive (%)	Negative (%)	
0 – 6	2 (1)	12 (6)	14 (7)
7 – 12	9 (4.5)	55 (27.5)	64 (32)
13-18	4 (2)	34 (17)	38 (19)
19-24	2 (1)	39 (19.5)	41 (20.5)
25-30	2 (1)	8 (4)	10 (5)
31-36	1 (0.5)	6 (3)	7 (3.5)
37-42	0 (0)	5 (2.5)	5 (2.5)
43-48	1 (0.5)	7 (3.5)	8 (4)
49-54	0 (0)	4 (2)	4 (2)
55-60	0 (0)	9 (4.5)	9 (4.5)
Total	21 (10.5)	179(89.5)	200(100)

P-Value = 0.776, $p > 0.05$

Table 2: Rotavirus infection in relation to sex of the studied children

SEX	Rotavirus Infection Status		Total (%)
	Positive (%)	Negative (%)	
Female	8 (4)	105 (52.5)	113 (55.5)
Male	13 (6.5)	74 (37)	87 (43.5)
Total	21 (10.5)	179 (89.5)	200 (100)

P – Value = 0.72, $p > 0.05$

Table 3: Rotavirus Infection in Relation to Signs and Symptoms presented

Signs & Symptoms	Rotavirus Infection Status		Total (%)
	Positive (%)	Negative (%)	
Diarrhea	3 (1.5)	91 (47)	94 (47)
Diarrhea & Fever	4 (2)	24 (12)	28 (14)
Diarrhea, Vomiting & Fever	11 (5.5)	19 (9.5)	30 (15)
Diarrhea % Vomiting	2 (1)	33 (16.5)	35 (17.5)
Vomiting	1 (0.5)	10 (5)	11 (5.5)
Vomiting & Fever	0 (0)	2 (1)	2(1)
Total	21(10.5)	179(89.5)	200(100)

P – Value = 0.01, < 0.05

Table 4: Rotavirus Infection in Relation to Marital Status of Parents /Guardian of the studied children

Marital Status	Rotavirus Infection Status		Total (%)
	Positive (%)	Negative (%)	
Married	20 (10)	164 (82)	184 (92)
Divorced	1 (0.5)	15 (7.5)	16 (8)
Total	21 (10.5)	179 (89.5)	200 (100)

P – Value = 0.563, $p > 0.05$

Table 5: Rotavirus Infection in Relation to Educational Status of Parents/Guardians

Educational Status	Rotavirus Infection Status		Total (%)
	Positive (%)	Negative (%)	
Primary	5 (2.5)	35 (17.5)	40 (20)
Secondary	10 (5)	79 (39.5)	89 (44.5)
Tertiary	2 (1)	39 (19.5)	41 (20.5)
Qur'anic	4 (2)	26 (13)	30 (15)
Total	21(10.5)	179(89.5)	200(100)

P-Value = 0.603, $p > 0.05$

Table 6: Rotavirus A Infection in Relation to Source of Drinking Water of the studied children

Source of Drinking Water	Rotavirus Infection Status		Total (%)
	Positive (%)	Negative (%)	
Tap Water	16 (8)	140 (70)	156 (78)
Well Water	4 (2)	12 (6)	16 (8)
Bore Hall	1 (0.5)	27 (13.5)	28 (14)
Total	21 (10.5)	179 (89.5)	200(100)

P – Value = 0.081, $p > 0.05$

Table 7: Rotavirus Infection in Relation to Distance of Toilet from Water Source

Distance of toilet from water source	Rotavirus Positive (%)	Infection Status Negative (%)	Total (%)
Far	18(9)	146 (73)	164 (82)
Near	3(1)	33 (16.5)	36 (18)
Total	21(10.5)	179 (89.5)	200(100)

P – Value = 0.640, $p > 0.05$

Table 8: Rotavirus Infection in Relation to Playing with Toys

Playing with Toys	Rotavirus Positive (%)	Infection Status Negative (%)	Total (%)
Yes	19 (9.5)	149 (74.5)	168 (84)
No	2 (1)	30 (15)	32 (16)
Total	21(10.5)	179 (89.5)	200 (100)

P – Value = 0.392, $p > 0.05$

Table 9: Rotavirus infection in relation to playing with other children

Playing with other children	Rotavirus Positive (%)	Infection Status Negative (%)	Total (%)
Yes	8 (4)	84 (42)	92 (46)
No	13 (6.5)	95 (47.5)	108 (54)
Total	21 (10.5)	179 (89.5)	200 (100)

P – Value = 0.442, $p > 0.05$

Table 10: Rotavirus Infection In relation to washing hands before meal

Washing hands before meal	Rotavirus Positive (%)	Infection Status Negative (%)	Total (%)
Yes	12 (6)	117 (58.5)	129 (64.5)
No	9 (4.5)	62 (31)	71(33.5)
Total	21(10.5)	179 (89.5)	200 (100)

P – Value = 0.456, $p > 0.05$

Table 11: Rotavirus infection in relation to consumption of food that require no cooking

Consumption of food that require no cooking	Rotavirus Positive (%)	Infection Status Negative(%)	Total (%)
Yes	7 (3.5)	79 (39.5)	86 (43)
No	14 (7)	100 (50)	114 (57)
Total	21 (10.5)	178 (89.5)	200 (100)

P – Value = 0.344, $p > 0.05$

Table 12: Rotavirus infection among the studied children in relation to type of feeding

Type of feeding	Rotavirus Positive (%)	Infection Status Negative (%)	Total (%)
Exclusive breast feeding	1 (0.5)	8 (4)	9 (4.5)
Bottle feeding	1 (0.5)	3 (1.5)	4 (2)
Mixed feeding	14 (7)	111 (55.5)	125 (62.5)
Normal feeding	5 (3)	57 (28.5)	62 (31)
Total	21 (10.5)	179 (89.5)	200 (100)

P - Value = 0.716, $p > 0.05$

DISCUSSION

The results of this study revealed a rotavirus infection rate of 10.5% (21/200) among children under 5 years of age in Kano State, which was comparable to 13.8% and 9% observed by Junaid *et al.* (2011) and Aminu *et al.* (2008) in Jos and Zaria respectively. On the other hand, Taqbo *et al.* (2014) and Kuta *et al.* (2013) reported high and low incidence rates of 56% and 6% in in Enugu and Kwara respectively. The differences in the incidence rates could be primarily explained by the differences in target population, age groups that were investigated in each study, place and duration of each study, seasonal variation and size of sample of each study. In addition, it could be due to the influence of environmental conditions, hygiene practice, and level of sanitation as documented in

studies by Atchison *et al.* (2010), Levy *et al.* (2009), Chandran *et al.* (2006) and Ansari *et al.* (1991)

The findings of this study further revealed that the rate of rotavirus infections decreases with increase in the ages of the children (though not consistent) as most of the lowest number of positive cases, or even negative samples were found among the older age groups. For example, the highest number of positive cases of 9/21 (42.9%) was found among children aged 7 – 12 months and the lowest rate of 0.5% was seen in two children, one aged 31 - 36 months and the other 13 - 48 months. Studies by Odimayo *et al.* (2008) have revealed similar observations and they explained that older children acquire protective immunity during repeated exposures to the virus and, therefore, subsequent infections are mild or

asymptomatic. Furthermore, the low incidence rate of 2/21 (9.5%) observed in the age group of 0 – 6 months may be due to passive immunity acquired from the mother which wades off after 6 months, or could be due to exclusive or high rate of breast feeding in the first 6 months which also protects the infants via passing of IgA antirotavirus antibodies to the infants as explained by Zarnani *et al.* (2004).

The incidence rate of rotavirus infection in this study did not differ significantly with regards to the sex of the studied children although 6.5% males were infected compared to 4.5% of females ($p > 0.05$). Thus, the male to female ratio of rotavirus infection in our study is 1.6:1 and was comparable with that of Junaid *et al.* (2011) who reported a ratio of 1.8:1 in Jos Nigeria. According to the WHO scientific group, the number of affected males was up to 20% higher than the number of females in some studies, but it was not known whether this was due to a greater susceptibility to rotavirus exposure in boys or a greater likelihood of parents of affected boys seeking medical care (Borade *et al.*, 2010; WHO 1980). Another reason could probably be due to the fact that males play with toys outside the room with other children.

It was further observed in this study that the rate of rotavirus infection was significantly associated with diarrhea as 99.5% of the children presented diarrhoea in addition to other symptoms ($p < 0.05$). This agrees with reports of Junaid *et al.* (2011) and Staat *et al.* (2002) who described that 90% percent of the children in their studies presented with diarrhea and/or vomiting and 91% had diarrhea (with or without vomiting and/or fever).

Demographic characteristics of parents/Guardian of the children were found to be insignificant as regards to the rate of rotavirus infection among the children, although the highest number of positive samples was found among 10/21 (47.6%) children whose parents/guardian had secondary education compared to 2/21 (9.5%) children whose parent had tertiary education ($p > 0.05$). Similarly, the highest number of infected children 20/21(95%) were from married couples.

Although the source of drinking water and location of toilet from the water source were not significantly associated with the rate of rotavirus infection among the children, yet 16/21 and 18/21 of the children positive for rotavirus were found to have tap water and have their toilets located far away from the water source. This shows that tap water is not safe for consumption when compared to the other sources of water (i.e. borehole and well) and could be attributed to possible contamination in the tap water either from the point of treatment or somewhere along the pipe and this possess a major health risk. Moreover, the

results of the study revealed that playing with toys by the children was more associated with rotavirus infection (although not significant) than the children playing with other children. This means that playing objects like toys should be hygienically suitable for use by the children.

Of particular interest in this study is the fact that no significant difference ($P > 0.05$) existed between children whose hands were washed before meal (12/21) and those who did not (4/21) in respect to the rate of rotavirus infection indicating that washing hands before meal has little effect in the transmission of rotavirus. This observation was however contrary to reports by Cairncross *et al.* (2010) who previously described sanitation as key in the control of infectious disease especially diarrhoeal diseases. Other researchers however, have shown that sanitation alone could not significantly improve the control of rotavirus diarrhea disease. This has been attributed to the fact that enteric viruses are more resistant to common water treatment processes than their coliform counterparts and are highly contagious as such improvements in water and sanitation were unlikely to be effective preventive measures of rotavirus disease, supporting the advocacy for mass vaccination programs (Huppertz *et al.*, 2008).

Although consumption of uncooked food by the children in this study was found to be insignificant, yet the difference in the infection rate could however be, attributed to the age of the children rather than a protective effect due to the nature of the foods. This is because foods that require no cooking are mostly eaten by the older children among whom prevalence of rotavirus infection is less common due to increase in age and possible previous exposure/infection to rotavirus. Furthermore, only one child on exclusive breastfeeding had rotavirus infections whereas the highest rate of (66.6%) was reported among 14 children who were on partial breastfeeding or mixed feeding (P -value = 0.716). The findings of this study support the observations made by Kramer and Kakuma (2012) who indicated that infants who are exclusively breastfed for the first six month are less likely to die of gastrointestinal infection than infants who switched from exclusive to partial breastfeeding at three to four months. Thus, WHO (2011) had earlier recommended that in the first six months of life, every child should be exclusively breastfed, with partial breastfeeding continued until two years of age.

CONCLUSION AND RECCOMENDATIONS

This study revealed an incidence of 10.5% rotavirus infection among children mostly aged 7-12 months. The infection was found to be significantly associated with diarrhea, fever and vomiting ($P < 0.05$).

Although insignificant ($p>0.05$), the sources of water especially tap water and playing with toys have been identified as the most predisposing factors of rotavirus infection. Exclusive breastfeeding was found to be effective in the prevention of rotavirus infection as 14/21 (66.6%) children were found to be infected on partial breastfeeding or mixed feeding and only 1/21 (4.7%) among the 9 exclusively breastfed children was infected ($p>0.05$). The study also identified ELISA method by Rotavirus Ag ELISA kit (2014), as a reliable method in the detection of rotavirus infection (100%), as all ELISA positive samples were also positive by the RT – PCR method by RT – PCR (Liferiver, 2012).

The study recommends the screening of rotavirus infection in diarrhoeal cases to be part of the routine laboratory tests in our hospitals. Children should be taught to observe strict personal hygiene including proper hand washing prior to engaging in some activities such as eating and playing with toys. Exclusive breastfeeding should be encouraged for infants from 0 – 6 month. The study also strongly advocates the concept of vaccination against rotavirus for children less than 2 years to be part of the national immunization program. Finally, further investigation should be carried out to determine the prevalent serotypes of rotavirus in Kano which could lead to the identification of appropriate and effective vaccine.

REFERENCES

- Ansari, S.A., Springthorpe, V.S. and Sattar, S.A. (1991): Survival and vehicular spread of human rotaviruses: possible relation to seasonality of outbreaks. *Reviews of Infectious Diseases*. **13** (3):448-61.
- Aminu, M., Ahmad, A.A., Umoh, J.U., Dewer, J., Esona, M.D. and Steele, A.D. (2008): epidemiology of rotavirus infection in North-western Nigeria. *Journal of tropical pediatrics*, **54** (5); 340-2
- Ansari, S.A., Sattar, S.A., Springthorpe, V.S., Wells, G.A. and Tostowaryk, W. (1988): Rotavirus survival on human hands and transfer of infectious virus to animate and nonporous inanimate surfaces. *Journal of Clinical Microbiology*. **26** (8):1513-8.
- Atchison C.J., Tam C.C., Hajat S., van Pelt W., Cowden J.M. and Lopman B.A. (2010). "Temperature-dependent transmission of rotavirus in Great Britain and The Netherlands". *Proceedings. Biological Sciences / the Royal Society* **277** (1683): 933–42.
- Bass, E.S., Pappano, D.A. and Humiston, S.G. (2007): *Rotavirus. Pediatrics in Review*, **28** (5):183-91.
- Borade, A., Bais, A., Bapat, V. and Dhongade, R. (2010): Characteristics of rotavirus gastroenteritis in hospitalized children in Pune. *Indian Journal of Medical Science* **64**:210-218.
- Butz, A.M., Fosarelli, P., Dick, J., Cusack, T. and Yolken, R. (1993). Prevalence of rotavirus on high-risk fomites in day-care facilities. *Pediatrics* **92** (2): 202–5.
- Cairncross, S., Hunt, C., Boisson, S., Bostoen, K., Curtis, V., Fung, I.C. and Schmidtn, W.P. (2010): Water, Sanitation and Hygiene for the prevention of diarrhea. *International Journal of Epidemiology*. **39** (1):i193-i205
- Centers for Disease Control and Prevention (2009): Prevention of rotavirus gastroenteritis among infants and children: recommendation of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recommendation and Report*; 58:1-25.
- Centers for Disease Control and Prevention (2007): *Epidemiology and Prevention of Vaccine-Preventable Diseases*. Atkinson W, Hamborsky J, McIntyre L, et al., eds. 10th ed. Washington, DC: Public Health Foundation; :295–306.
- Centers for Disease Control and Prevention (2006): Prevention of rotavirus gastroenteritis among infants and children. Recommendations of the Advisory Committee on Immunization Practices (ACIP). *The Morbidity and Mortality Weekly Report* 55(RR-12):1–16.

Contribution of Authors

The study was conceived and designed by Aminu, A.I. and Muhammad, A. and performed by Muhammad, A. Generated data was analyzed, interpreted and presented by Aminu, A.I., Muhammad, A. and Muhammad, Y.

Conflict of Interest Statement

None of the authors had any financial relationship with a commercial entity such as manufacturers of the test kits used in the study. Thus, the commercial entities as well as the respective hospitals from where samples of the study were generated had no interest with regards to subjects of the study and outcome of the study. Furthermore, no outside funding was received for the study.

Acknowledgement

We wish to acknowledge Kano state hospitals management board for the approval given to conduct the study, and also the technical support of Magaji Ahmadu of Microbiology Laboratory, Aminu Kano Teaching Hospital, who contributed immensely in the processing and confirmation of the positive samples of the study.

- Chandran, A., Heinzen, R.R., Santosham, M. and Siberry, G.K. (2006): Nosocomial rotavirus infections: a systematic review. *Journal of Pediatrics*, **149** (4):441-7.
- Chang, H.G., Glass, R.I., Smith, P.F., Cicirello, H.G., Holman, R.C. and Morse D.L. (2003): Disease burden and risk factors for hospitalizations associated with rotavirus infection among children in New York State, 1989 through 2000. *Pediatric Infectious Diseases Journal*, **22**:808–814.
- Dennehy, P.H. (2000): Transmission of rotavirus and other enteric pathogens in the home. *Pediatric Infectious Disease Journal*, **19** (10): S103–5
- Dennehy, P.H., Nelson, S.M., Crowley, B.A. and Saracen, C.L. (1998). Detection of rotavirus RNA in hospital air samples by polymerase chain reaction (PCR). *Pediatric Research*, **43**:143A.
- DRG (2013). Rotavirus Group A Ag ELISA Kit User Manual (online). Available: <http://www.drg-international.com>, 841 Mountain Avenue, Springfield, NJ 07081 USA
- Dzikwi, A.A., Umoh, J.U., Kwaga, J.K.P., Ahmad, A.A., deBeer, M. and Steel, A.D. (2008): electrophoretotypes and sub-groups of Group A rotavirus circulating among diarrhoeal children in Kano. *Annals of African Medicine*, **7** (4):163-7
- Glass, R.I., Parashar, U.D., Bresee, J.S., Turcios, R., Fischer T.K., Widdowson, M.A., Jiang B., and Gentsch, J.R. (2006): Rotavirus vaccines: current prospects and future challenges. *Lancet*, **368** (9532): 323–32.
- Grimwood, K. and Lambert, S.B. (2009): Rotavirus vaccines: opportunities and challenges. *Human Vaccines*, **5** (2): 57–69.
- Huppertz, H.I., Salman, N. and Giaquinto, C. (2008): Risk factors for severe rotavirus gastroenteritis *Pediatric Infectious Disease*, **27** (1):11 – 119.
- Junaid, S.A., Umeh, C., Olabode, A.O. and Banda, J.M. (2011): Incidence of rotavirus infection in children with gastroenteritis attending Jos university teaching hospital, Nigeria. *Virology Journal*, **8**: 233.
- Kirkwood, C.D. (2010): Genetic and antigenic diversity of human rotaviruses: potential impact on vaccination programs. *The Journal of Infectious Diseases*, **202** (Suppl): S43–8.
- Kotloff, K.L., Nataro, J.P., Blackwelder and W.C. (2013): Burden and etiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. *Lancet*, **382**:209–222.
- Kramer, M.S. and Kakuma, R. (2012). Optimal duration of exclusive breastfeeding. *The Cochrane database of systematic reviews* **8**: CD003517. *AdvExp Med Bio*, 554: 63–77.
- Kuta, F.A., Uba, A., Nimzing, L., and Damisa I.D. (2013): Molecular identification of rotavirus strains associated with diarrhea among children in Kwara state, Nigeria. *Bayero Journal of Pure and Applied Sciences*, **6** (2): 23 – 26
- Levy K., Hubbard A.E. and Eisenberg J.N. (2009). "Seasonality of rotavirus disease in the tropics: a systematic review and meta-analysis". *International Journal of Epidemiology*, **38** (6): 1487–96.
- Leung, A.K., Kellner, J.D. and Davies, H.D. (2005): Rotavirus gastroenteritis. *Advances in Therapy*, **22** (5): 476–87.
- Liferiver Shanghai ZJ Bio-Tech Co., Ltd. (2012). Rotavirus (Group A) Real Time RT-PCR Kit User Manual, (online). Available: <http://www.liferiver.com.cn>, trade@liferiver.com.cn, Xinjunhuan road, Pujiang Hi-tech Park Shanghai China (issue date July 2012).
- Nelson, E.A., Bresee, J.S., Parashar, U.D., Widdowson, M.A. and Glass, R.I. (2008). Rotavirus epidemiology: the Asian Rotavirus Surveillance Network. *Vaccine*, **26** (26):3192-6.
- Odimayo, M.S., Olanrewaju, W.I., Omilabu, S.A. and Adegboro B. (2008): Prevalence of rotavirus-induced diarrhea among children under 5 years in Ilorin, Nigeria. *Journal of Tropical Pediatrics*, **54** (5):343-6.
- Parashar, U.D., Gibson, C.J., Bresse, J.S. and Glass, R.I., (2006): Rotavirus and severe childhood diarrhea. *Emerging Infectious Disease*, **12** (2):304–6.
- Parashar, U.D., Hummeiman, E.G., Bresse, J.S., Miller, M.A. and Glass, R.I. (2003): Global illness and deaths caused by rotavirus disease in children. *Emerging Infectious Diseases*, **9**: 565-572.
- QIAGEN QIAamp Viral RNA mini kit (2011). Viral RNA Extraction manual, (online). Available: <http://www.qiagen.com>: Skelton House Lloyd Street, North Manchester.
- Rheingans, R.D., Heylen, J. and Giaquinto, C. (2006): Economics of rotavirus gastroenteritis and vaccination in Europe: what makes sense? *Pediatrics Infectious Diseases. Journal*, **25** (1 Suppl): S48–55.
- Rodrigo, C., Salman, N., Tatochenko, V., Mészner, Z. and Giaquinto, C. (2010): Recommendations for rotavirus vaccination: A worldwide perspective. *Vaccine*, **28** (31): 5100–8.
- Samukaddam S.B. and Gard S.G. (2006). Validity of Assumption while Determining sample size. *Indian journal of community medicine*. **29**(2)
- Simpson, E., Wittet, S., Bonilla, J., Gamazina, K., Cooley, L. and Winkler, J.L. (2007): Use of formative research in developing a knowledge translation approach to rotavirus vaccine introduction in developing countries. *BMC Public Health*, **7**: 281.

- Staat, M.A., Azimi, P.H., Berke, T., Roberts, N., Bernstein, D.I., Ward, R.L., Pickering, L.K. and Matson, D.O. (2002): Clinical presentations of rotavirus infection among hospitalized children. *Pediatric Infectious Disease Journal*, **21** (3):221-7
- Tagbo, B.N., Mwenda, J.M., Armah, G., Obidike, E.O., Okafor, U.H., Oguonu, T., Ozumba, U.C., Eke, C.B., Chukwubuike, C., Edelu, B.O., Ezeonwu, B.U., Amadi, O., Okeke, I.B., Nnani, O.R., Ani, O.S., Ugwuezeonu, I., Benjamin-Pujah, C., Umezinne N., Ude N., Nwodo C., Ezeonyebuchi MC., Umesie E., Okafor V., Ogude, N., Osarogborum, V.O., Ezebilo, S.K., Goitom, W.G., Abanida, E.A., Elemuwa, C. and Nwagbo, D.F. (2014): Epidemiology of rotavirus diarrhea among children younger than 5 years in Enugu, South East, Nigeria. *Pediatrics Infectious Diseases Journal*, **33** Suppl 1:S19-22.
- Tate, J.E., Burton, A.H., Boschi-Pinto, C., Steele, A.D., Duque, J. and Parashar, U.D. (2012): 2008 estimate of worldwide rotavirus-associated mortality in children younger than 5 years before the introduction of universal rotavirus vaccination programmes: a systematic review and meta-analysis. *Lancet Infectious Disease*, **12**(2):136–141.
- Wobudeya *et al.* (2011). World Health Organization (2011). Global strategy for infant and young child feeding. The optimal duration of exclusive breastfeeding, in, Geneva.
- World Health Organization (2008): Global networks for surveillance of rotavirus gastroenteritis, 2001–2008. *Weekly Epidemiological Record* **83** (47): 421–428.
- Zarnani, A.H., Modarres, S.H., Jadali, F., Sabahi, F., Moazzeni, S.M. and Vazirian, F. (2004): Role of rotaviruses in children with acute diarrhea in Tehran, Iran. *Journal of clinical virology*, **29** (3):189-193.