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ORIGINAL ARTICLE

Molecular Detection of Human Norovirus among Children (0-5 Years) Attending Selected Hospitals in Kano Metropolis, Kano State, Nigeria

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ABSTRACT

Background: *Norovirus has been reported as the main cause of acute diarrhea worldwide after rotavirus. But its incidence in developing countries such as Nigeria has been underreported.* ***Objectives****: This study aimed to detect human Norovirus genogroups I, II and IV in stools of children (0-5 years) with diarrhea in three selected Hospitals (Muhammad Abdullahi Wase Specialist Hospital, Murtala Muhammad Specialist Hospital, and Hasiya Bayero Pediatric Hospital) in Kano, Nigeria, estimate the prevalence of Norovirus infection, determine the socio-demographic characteristics and other factors and examine the clinical profile of the study children in relation to their infection status.* ***Methodology:*** *Two hundred (200) diarrheic fecal samples were collected from 0-5 years children between June-October 2021. The samples were examined for NoV using one-step real time reverse transcription-Polymerase chain reactions (RT-PCR) technique.* ***Results:*** *In this study, the total prevalence of Norovirus infection was found to be 21%, with Genogroups II having the highest frequency of 25 (12.5%), followed by Genogroup I having 11(5.5%), and Genogroup IV with the lowest frequency; 6(3.0%). Our investigation identified co-infections of three Norovirus genogroups: I and II (0.5%), I and IV (0.5%), and I, II and IV 2(1.0%).* ***Conclusion:*** *According to this study, Norovirus Genogroup II is the most common strain circulating in Kano, Nigeria, and it has been established that NoV is a relevant cause of pediatric diarrhea. However, there isn't any evidence linking the Norovirus to any other risk factors. In order to stop the spread of NoV, it is essential to keep the environment clean and further research is needed with more samples to detect the link between risk factors and the presence of the virus.*

INTRODUCTION

Acute gastroenteritis (AGE) is a common cause of morbidity and mortality in children, especially among those below the age of one year[1]. The World Health Organization (WHO) estimated that 1.5 million under-five children die from diarrheal diseases every year, with almost half of them in Africa[2]. Data shows that Norovirus accounts for about one-fifth of AGE cases globally[3], while in countries that have introduced Rotavirus vaccines, Norovirus has become the leading cause of medically-attended AGE[4].

Noroviruses (NoVs) belong to the family *Caliciviridae*. They are non-enveloped viruses with icosahedral capsid symmetry that contain single-stranded, positive-sense, RNA genome of about 7,500 nucleotides in length[5]. The genome of Noroviruses has three open reading frames (ORF): ORF1 encodes the viral RNA-dependent RNA polymerase (RdRp), ORF2 encodes the viral protein (VP1) and ORF3 encodes the minor capsid protein (VP2)[6]. The VP1 consists of two main domains: the inner shell and the protruding arm which is further divided into the P1 and P2 subdomains[7]. The surface-exposed P2 subdomain is hypervariable and it contains the main antigenic histo-blood group antigen (HBGA) binding sites[8]. These factors play an integral role in determining susceptibility to NoV infection[9].

Noroviruses are mostly transmitted by the fecal-oral route, which includes direct person-to-person transmission as well as indirect transmission through contaminated food, drink, or environmental surfaces[10]. Following an average incubation period of 24 to 48 hours, symptoms and signs of NoV infection start to appear, which include diarrhea, projectile vomiting, fever, abdominal cramps and dehydration[8]. Severe disease is most often observed in young children below

five years of age[11], in elderly (>65 year of age) [12], and immunocompromised individuals[13].

Noroviruses are categorized genetically in to at least ten accepted genogroups (GI to GX) and 49 confirmed genotypes, as well as two tentative genogroups (GNA1 and GNA2) and three proposed genotypes[14]. Genogroups GI, GII, GIV, GVIII and GIX (previously GII.15) infect humans and cause acute gastroenteritis, with GII detected most frequently in clinical surveillance studies throughout the world[15].

The predominant strain causing both outbreaks and sporadic cases of gastroenteritis is genogroup II genotype 4 (GII.4)[16]. Despite the broad genetic diversity, the GII.4 has been the major circulating genotype in community and health-care settings since 1996 with periodic emergence of novel GII.4 variants after every 2-4 years[17]. Infection, mainly with GII.3 and GII.4, have also been reported in non-secretors phenotype due to the absence of HBGAs expression involved in the attachment of NoV to intestinal cells[18]. Other genotypes like GII.17 and GII.2 are emerging and have become predominant in certain regions of the world[19, 20].

METHODOLOGY

The present study was conducted at three major referral hospitals in Kano (Muhammad Abdullahi Wase Specialist Hospital, Murtala Muhammad Specialist Hospital, and Hasiya Bayero Paeditric Hospital).

Kano is located at latitude 12^0 3' north and longitude $8^0$31'east[21] and is one of the states with the highest population in Nigeria estimated at 14,363,776 projected at 2.6% annual growth from the 2006 census[22].

Study Population:

The study populations were children (0-5 years) of age with complaints of diarrhea with or without other complains who presented to the selected hospitals within the study period. Only children between 0-5 years passing loose or watery stools more than 2 times in 24 hours with or without other clinical symptoms whose mothers/caregivers consented were included. While those in the same age range passing loose or watery stools more than two-times in 24 hours whose mothers/caregivers denied consents were excluded.

Sampling techniques:

The study was a multicenter hospital-based cross-sectional in design. Socio-demographic, family, and clinical history of the participants were obtained using a pre-designed semi-close-ended questionnaire. Information was either collected from the mothers or the caregivers. The appropriate sample size was determined using the formula proposed by Lwanga and Lemeshow, the total sample size was determined as 113 to improve precision, the sample size was increased to 200.

Sample Collection:

Two hundred (200) diarrheic stool samples were collected from the children between June and October 2021, using sterile universal containers and stored at -20ºC until use.

Ethical Approval:

Ethical clearance was sought for and obtained from the research and ethics committee of Kano state ministry of health, with the following code; NHREC/17/03/2018.

Preparation of Stool Samples:

Ten milliliter (10 mL) processing buffer (containing 9 mL phosphate buffered saline and 1mL chloroform), was added in to the stool sample. The mixture was shaken vigorously for 20 minutes at 2000 revolution per minute (rpm) and the suspension clarified by centrifugation at 4000 rpm for 20 minutes. The supernatants were used for the viral RNA extraction and purification[23].

Norovirus Genomic RNA Extraction

The genomic viral RNA was extracted using Quick-DNA/RNA kit (Zymo-Research, USA), in accordance with the manufacturer's instructions. The pure eluted RNA was eluted in eppendorf tube and store at -20ºC until use.

Primer Sets and Probes used in the Study

The primer sets and probes used for NoV GI and GII detection were adopted from Kageyama *et al*[24], while those for NoV GIV detection were adopted from Yan *et al* [25] as shown in Table 1. The primer sets and probes were synthesized by Inqaba Biotec West Africa Limited.

The final concentrations of primers (COG1F/R, COG2F/R, and NVG4F/R) and probes (RING1(a)-TP, RING2-TP, NVGPg) for NoV GI, GII, and NoV GIV detection were 0.8 µ L and 0.2 µ L, respectively[24].

Table 1: Primer sets and Probes used in the Study

Target	Name	Sequence	Sense	Location
GI	COG1-F	CGYTGGATGCGNTTYCATGA	+	5291[b]
	COG1-R	CTTAGACGCCATCATCATTYAC	-	5375[b]
	RING1(a)-TP[e]	AGATYGCGATCYCCTGTCCA	-	5340[b]
GII	COG2-F	CARGARBCNATGTTYAGRTGGATGAG	+	5003[c]
	COG2-R	TCGACGCCATCTTCATTCACA	_	5100[c]
	RING2-TP[f]	TGGGAGGGCGATCGCAATCT	+	5048[c]
GIV	NVG4-F	TGGATGCGRTTCTCNGACYT	+	4971[d]
	NVG-R	GACGCCATCWTCATTYAC	_	5072[d]
	NVG-P[g]	GATCGCRATCTCGCTCCCGA	+	5021[d]

a: Degenerate bases in the primers and probes: Y, C or T; R, A or G; W, A or T; B, not A; N, any.
b: Nucleotide position on Norwalk virus complete cds (M87661) of the 5' end; **c**: Nucleotide position on Lordsdale virus complete genome (X86557) of the 5' end; **d**: Nucleotide position on Norovirus Hu/GIV.1/LakeMacquarie/NSW268O/2010/AU complete genome (JQ613567) of the 5' end; **e**: Probes labeled with HEX-TAMRA (5-3); **f**: Probes labeled with FAM-TAMRA (5-3); **g**: Probes labeled with CY3-TAMRA (5-3). GI, II and IV stand for Nov genogroups I, II and IV.

Assay Mix Preparation

The master mix used was obtained from New England Biolab and the mixture contains; Luna Universal One-Step real time Reaction Mix (5 µL), Luna Warmstart RT Enzyme Mix (0.5 µL), 0.8 µL forward and reverse primers, 0.2 µL probe, 5 µL RNA template, and 7.7 µL nuclease-free water. Except for the RNA Template, all of the components were pipetted and properly mixed.

After aliquoting the assay mixture into Mic qPCR tubes, the RNA Template was added and sealed, and the tubes loaded in to the thermocycler.

One-Step Real-time RT-PCR Assay (Amplification of the viral RNA)

The NoV genomic RNA was amplified using the Luna Universal One-Step real-time RT-PCR Kit on a Magnetic Induction Cycler (Mic qPCR) BioMolecular System in a 20 µL total reaction volume. Multiplex RT-PCR was used for Nov genogroups I and II (table 2, fig 1).

Table 2: Thermalcycling Condition for Norovirus One-Step Real-time RT-PCR Assay

Cycle steps	Temperature (^{0}C)	Time	Cycles
cDNA synthesis	55	10 minutes	1
PCR Activation	95	60 seconds	1
Denaturation	95	10 seconds	
Annealing and Amplification	60	30 seconds	45

The One-Step multiplex real-time RT-PCR assay was established by combining the two GI and GII primers and probes with their respective optimal reaction concentrations of 20µL and co appropriate reaction conditions. For GIV, the assay was done separately using the same protocols applied to GI and GII.

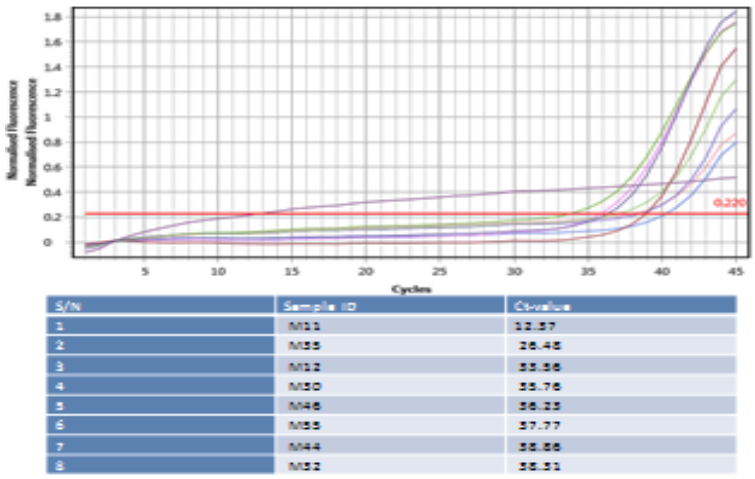

Fig. 1: showing the amplification plot for some samples using one-step real-time RT-PCR.

Data Analysis

The data generated from the study were prepared in Excel spreadsheet and then exported into IBM Statistical Package for Social Sciences (SPSS) version 22 for statistical analysis, demographic data, medical history and risk factors were analyzed with descriptive statistics and reported in frequency tables and figures. Associations between variables were assessed using Chi-square test. A *P-value* <0.05 was considered statistical significant.

Prevalence of Norovirus Infection among the Study Children

The overall prevalence of human Norovirus infection in this study was 21% (42/200), with Genogroup II having the highest prevalence of 59.5% (25/42), followed by Genogroup I 26.2% (11/42), and Genogroup IV with the lowest prevalence 14.3% (6/42) (Table 3).

The estimated prevalence of co-infection was 2.4% (1/42) for each of genogroups I and II and I and IV, respectively, while co-infection with all the three genogroups (I, II and IV) was found in two children (4.7%), giving a total prevalence of 9.5% for the co-infections.

RESULTS

Table 3: Prevalence of Human Norovirus Infection among the Study Children

Overall		**Number tested = 200**	
		42	21
Genogrous		**Number positive**	**Percentage (%)**
	Genogroup I	11	26.2
	Genogroup II	25	59.5
	Genogroup IV	6	14.3
Overall		**Number tested = 42**	
		4	**9.5**
Genogroups		**Co-infection**	**Percentage (%)**
	Genogroup I and II	1	2.4
	Genogroup I and IV	1	2.4
	Genogroup I, II and IV	2	4.7

Association of Norovirus Infection and Socio-demographic Profile of the Children

The study findings revealed that children below 6-months had the highest prevalence (27.3%; 6/22), followed by those in the age range 6-12 months 14/55 (25.5%), while the least was among those older than 24 months (16.7%; 3/18). However, there was no variation in the prevalence of the infection with respect to gender (M = 21.2%: 22/104 *versus* F = 20.8%: 20/96), and no statistically significant difference in the distribution of the infection across all the variables tested (Table 4).

Association of NoV Infection with Family and Social History of the Study Children

Our findings revealed higher infection rate among children from the urban settlements (22.8%), those who played with toys (21.3%), children not exclusively breastfed (21.6%) and used feeding bottles (22%), whose family used sachet water as sources of drinking water (31.4%), and those whose mothers/caregivers had non-formal/primary education (21.5%). Similarly, the prevalence was higher among children not attending daycare centers (22.7%), and those that had dogs in or near their households (25%). In addition, the prevalence was higher among children whose mothers employed the services of house help (33.3%). Majority (35/42) of the positive cases had no recent contact with a family member with diarrhea, and did not visit a sick person with diarrhea (33/42), respectively. Lastly, the prevalence was higher among children whose mothers do not boil water for family use (21.1%) (Table 5a and b).

Table 4: Association of Norovirus infection and Socio-demographic Factors of the Study Children

Variables	Norovirus infection		χ^2	*p-value*
	No. tested	No. positive (%)		
Age (months)				
<6	22	6 (27.3)	1.917	0.59
6 – 12	55	14 (25.5)		
13 – 24	105	19 (18.1)		
>24	18	3 (16.7)		
Gender				
Male	96	20 (20.8)	0.003	0.956
Female	104	22 (21.2)		
Religion				
Islam	197	42 (21.3)	0.810	1.000
Christianity	3	0 (0.0)		

Key: χ^2 = Chi-square.

Table 5a: Association of NoV Infection, Family and Social History of the Children

Variable	Norovirus infection		χ^2	*p-value*
	No. tested	No. positive (%)		
Place of residence				
Rural	42	6 (14.3)	1.445	0.289
Urban	158	36 (22.8)		
Maternal/caregiver Education				
Non-formal/primary	102	22 (21.5)	0.041	0.864
≥ Secondary	98	20 (20.4)		
Maternal occupation				
Full-time housewife	119	26 (21.8)	0.128	0.860
Non-fulltime housewife	81	16 (19.7)		
Type of family				
Extended family	31	7 (22.6)	0.055	0.812
Nuclear family	169	35 (20.7)		
Number of individuals per room				
≤4 persons	120	26 (21.6)	0.080	0.860
>4 persons	80	16 (20)		
CCC attendance				
Daycare/Sch/Nurs. home	42	6 (14.3)	1.445	0.289
None	158	36 (22.7)		
Child play with toys				
Yes	141	30 (21.3)	0.022	1.000
No	59	12 (20.3)		
Presence of dog near/in the house				
Yes	52	13 (25)	0.678	0.432
No	148	29 (19.5)		
FSDW				
Sachet water	35	11 (31.4)	0.41	0.843
Non-sachet water	165	31 (18.8)		
Exclusive breast feeding				
Yes	29	5 (17.2)	0.289	0.806
No	171	37 (21.6)		

Key: CCC = Child care center; FSDW = Family source of drinking water; Nurs = Nursing; Sch = School.

Table 5b: Association of NoV Infection and Family and Social History of the Study Children cont…

Variable	Norovirus infection		χ^2	*p-value*
	No tested	No Positive (%)		
Use feeding bottle for the child				
Yes	50	11 (22)	0.040	0.843
No	150	31 (20.6)		
Who does the house cleaning				
Mother	182	36 (19.7)	1.814	0.222
House help/children	18	6 (33.3)		
Contact with someone with diarrhea in the household				
Yes	32	7 (21.8)	0.018	1.000
No	168	35 (20.8)		
Contact with someone with diarrhea outside the household				
Yes	35	7 (20)	0.026	1.000
No	165	35 (21.2)		
Visited sick person				
Yes	39	9 (23)	0.126	0.827
No	161	33 (20.4)		
Wash hands with soap after returning from toilet or changing diaper				
Yes	152	33 (21.7)	0.193	0.839
No	48 (24.0)	9 (18.7)		
Wash fruits and vegetables with salt and clean water before serving use				
Yes	146	30 (20.5)	0.067	0.846
No	54 (27.0)	12 (22.2)		
Wash hands with soap and water before food preparation				
Yes	91	16 (17.5)	1.176	0.300
No	109	26 (23.8)		
Use PPE before cooking				
Yes	3	2 (66.6)	3.829	0.112
No	197	40 (20.3)		
Boil water before use				
Yes	16	3 (18.7)	0.053	1.000
No	184	39 (21.1)		

Key: PPE = Personal protective equipment.

Association of the Norovirus Infection and Clinical History of the Study Children

Table 6; depicts the clinical history of the study children in relation to the Norovirus infection. It can be deduced that (83.3%) of those with the Norovirus infection presented with fever, suggesting that fever is a cardinal symptoms. Vomiting is another important clinical presentation of Norovirus infection as it was reported by 59.5% of the children with the positive test result. Similarly, use of antibiotics could be a predisposing factor to the Norovirus infection as (71.4%) reported using some form of antibiotics prior to the commencement of the diarrhea. More importantly, there was a statistically significant association between hospital admission and the Norovirus positivity (p = 0.041), although it may be difficult to conclude that the infection was hospital-acquired in the study children, but it could be is a possibility.

Table 6: Association of Norovirus Infection and Clinical History of the Study Children

Variables	Norovirus infection		χ^2	p-value
	No Tested	Positive (%)		
Presence of fever				
Yes	177	35 (83.3)	1.394	0.276
No	23	7 (16.7)		
Frequency of stooling				
≤3 times/day	93	21 (50.0)	0.262	0.728
>3 times/day	107	21 (50.0)		
Duration of diarrhea				
24 – 48 hours	29	7 (16.7)	0.201	0.628
>48 hours	171	35 (83.3)		
Presence of vomiting				
Yes	122	25 (59.5)	0.049	0.860
No	78	17 (40.5)		
Currently on admission				
Yes	141	29 (69.0)	0.054	0.850
No	59	13 (31.0)		
Developed diarrhea on admission				
Yes	66	8(19)	4.681	0.041*
No	134	34(81)		
Medication used				
Antibiotics	145	30 (71.4)	0.521	0.800
Other medications	47	12 (28.6)		
Background illness				
Yes	11	1 (2.4)	0.995	0.464
No	189	41 (97.6)		
Completed routine immunization				
Yes	127	24 (57.1)	0.927	0.370
No	73	18 (42.9)		

Key: χ^2 = Chi-square.

DISCUSSION

Norovirus is being reported as the main cause of acute diarrhea after Rotavirus in all paediatric groups, both in the developed and the developing countries.

The prevalence of NoV among children (0-5 years) was found to be 21% in the study area; this is similar to the findings of Osazuwa *et al.*[26] and Ahmad *et al.*[27] with a prevalence of 22.9% and 20.0% respectively, all from Nigeria. However, the finding of this study is lower than that obtained in Lagos, Nigeria, by Ayolabi *et al.*[28] who reported a prevalence of 37.3%. Similarly, the results were relatively higher than the reports of other studies from parts of the country; 3.6%[29] in Edo State, 6.7%[30] from the North-east, 8.0%[31] from Lagos state, 12.0%[32] from South-south, 15.0%[33] from Niger Delta zone, 10.7%[34], and 6.7% from Bayelsa state[26].

Moreover, studies conducted in other countries including from Asia revealed higher prevalence rates; 33.3% from Indonesia[35], 26.4%[36] from Tianjin, China, and 27%[37] in Brazzaville, Republic of Congo.

The observed differences can be attributed to variations in social and cultural practices, population size, study individuals, personal and environmental hygienic practices as well as climatic factors, sample size, and the diagnostic method used for the NoV detection. It is important to note that, the current study employed one-step real-time RT-PCR, which is the gold standard test for Norovirus detection in all clinical samples by most public health and research laboratories.

With regards to genogroups detection rates, in our study, NoV genogroups I, II, and IV were detected with a 59.5%, 26.2% and 14.3% for genogroup II, I, and IV, respectively. This indicates that NoV genogroup II are the most circulating among the study participants, and possibly in Kano state and the country at large. This finding is consistent with other results from Nigeria including that of Osazuwa *et al.*[32] in South-south and Ayolabi *et al.*[28] from Lagos state, and also from other countries like Indonesia[35] and The Democratic Republic of Congo[37]. Interestingly, our result is in agreement with the global findings, indicating genogroup II as the most prevalent genogroup in humans. Our study also identified some co-infections within genogroups; I and II (2.4%), I and IV (2.4%), and I, II and IV (4.7%). This is in line with the findings of Ayolabi *et al.*[28] who reported 19% mixed infection of NoV genogroups I and GII.

Concerning Age, the majority of Norovirus infections in this study were found in children between the ages of 0-6 months 6(27.3%) (P=0.59), which is similar to the work of Trang *et al*[38] in Nigeria, Louya *et al*[37] in Brazzaville, Republic of Congo and Oluwatoyin *et al*[39] in Vietnam. Inpatient settings and lower-income nations also exhibit younger age distributions, according to Shioda *et al*[40]. This demonstrated that antibodies produced by mothers during breastfeeding are insufficient to protect against NoV infection, in addition, kids start learning how to crawl at 3 to 4 months old, so there's a lot of temptation for them to pick up things on the ground and put them in their mouth. These infected materials picked up from the ground would then act as a possible mechanical vector because the virus is transmitted via the fecal-oral route.

In contrast, the findings obtained by Ayolabi *et al*[28] and Arthiyya *et al*[35]; found that the majority of infections occurred in populations of children between the ages of 6 and 12 months and 6 to 23 months, respectively. Similarly, Louya *et al*[37] and Fang *et al*[36] found that Norovirus infection was found exclusively in children aged less than 24 months with a higher prevalence in the age group of 7-12 months. According to research by Babalola *et al*[31] in Ondo state, Nigeria, Norovirus was not found in infants less than 1 year old, however, it was found in 20% of infants between 25-30 months. Oyinloye *et al*[30] discovered comparable research that demonstrates the incidence of Norovirus throughout the three states, with children under the age of 2 being affected in a larger proportion (24/40). Their findings may be due to protection from maternal antibodies during breastfeeding for infants of < 6 months old.

The distribution of sex in this study revealed that the number of females positive for Norovirus infection was slightly higher than males (22/42; 20/42 respectively) The slight difference obtained in this study implies that females are more likely to be infected than males which are similar to what was obtained by Louya *et al*[37]. In contrast to Babalola *et al*[31] finding showed that gender does not play a role in Norovirus infection as male and female children under the age of five make up the majority of those who are infected. Oyinloye *et al*[30] also found that the prevalence of Norovirus in males was found to be higher than that of their female counterparts.

Although there was no statistically significant correlation (p>0.05) between Norovirus infection and any of the family and social history of the study children, Most of the children with Norovirus infection investigated in this study were from the urban area 36(22.9%) which is similar to what was obtained by Louya *et al*[37], However, it's in contrast with previous studies[10]. The reason may be due to congestion and improper hygienic practices by their parents /caregivers that dwell in urban areas. Corresponding to this, the rates of Norovirus infection were slightly among parents/caregivers with non-formal/primary education 22(21.5%) than among those with higher education level 20(20.4%), this observation may be a result of parents/caregivers' ignorance of the advantages of standard personal hygiene practices like washing hands after using the restroom before handling or caring for their children. Contrary to expectations, Oyinloye *et al*[30] data showed that parents of children with non-formal education had a lower frequency of Norovirus infection than parents with higher education (19.8%). In the current study, the study population shows that; there was no statistically significant correlation between education level and Norovirus infection.

In agreement with the findings of Oyinloye *et al*[30] and Osazuwa *et al*[26], the results of this study show that the infection rates were greater among parents whose children played with toys, parents/caregivers who did not practice exclusive breastfeeding and those who did not boil water before using it. However, this may be because children's toys are frequently dropped to the ground, picked up with bare hands, rubbed on clothing (which is occasionally put in mouth), or even put such toys directly in their mouths; these actions cause infection through the oral route and exclusive breastfeeding was highly protective against NoV infection. In contrast, there was no link in our study related to water infections.

This study also revealed that there is no statistical association (p>0.05) between any of the other clinical profiles of the study children, hence, in our study, it was found that there was an association between Norovirus infection and the development of child diarrhea while in admission (P=0.041), this demonstrates that Nov infection was strongly associated with close contacts with sick patients. However, in contrast to the findings of Louya *et al*[37] found no distinct factor that could be substantially related to Norovirus infection.

CONCLUSION

The present study showed that Norovirus is an important cause of diarrhea among children 0-5 years of age.

The prevalence of Norovirus infection in stool samples collected from the patients with clinical symptoms of infection for analysis at three selected hospital was 21% and Norovirus GII was significantly dominant compared to Norovirus GI and GIV.

The risk factor of having Norovirus infection increased while visiting patients that had the virus. While maintaining a clean environment is crucial to preventing the spread of NoV in hospital settings, more development is required to reduce the risk of transmission.

Declarations:

Consent for publication

Not applicable

Availability of data and material

Data are available upon request

Competing interests

The author(s) declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article none.

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