ISSN NO. 2320-5407

*Journal homepage:http://www.journalijar.com*

*INTERNATIONAL JOURNAL OF ADVANCED RESEARCH*

**RESEARCH ARTICLE**

# The incidence of Norovirus compared with Rotavirus in Baghdad City

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## *Manuscript Info* *Abstract*

***Manuscript History:***

Received: 11 November 2013
Final Accepted: 25 November 2013
Published Online: December 2013

***Key words:***
Norovirus ,Rotavirus, Gastroenteritis, co-infection

*Norovirus* are the commonest cause of outbreaks of non-bacterial acute gastroenteritis, and it is the most commonly recognized food borne viral infection and second only to *Rotavirus* as a cause of severe diarrhea in children . This study describes, for the first time incidence of *Norovirus* in Baghdad Between May 2012 and May 2013, 252 stool samples were collected from children31(12.30%) cases of males and 27(10.71%) cases of females) in the age group under 5 years from Baghdad Hospitals. Norovirus genogroups detected in fecal specimens using real time reverse transcription PCR.81 samples were identified as Norovirus ,and 62 as *Rotavirus*.Recurrence ofNorovirus in males were more than in females.Study appeared high incidence Norovirus during August 66.6% ,Mars 55% ,June 50% and January 40.9% and June ,and dominated NV GII genogroup on VN GI genogroup.*Norovirus*NVGIand NVGII co-infections wasrepresented (3.5%)and Rotavirus–Norovirus co-infection was represented ( 3.5% )of all cases ,with more severe symptomsandlong duration in Norovirus-Rotavirus co-infection.



Noroviruses are the commonest cause of

## Introduction

Noroviruses (NoVs) are not only the leading causative agents of outbreaks of acute viral gastroenteritis worldwide in people of all ages, but also the second most common viral etiological agents of severe childhood gastroenteritis after *Rrotavirus* (Patel *et al*.,2008;Glass *et al*., 2009), and the principle cause of foodborne illness in Europeand the United States (Kronema*net al*., 2009; Philips *et al*., 2010). The number of estimated cases in the United States was recently revised to 5.5 million annually (Scallan*et al*.,2011), while England has an estimated 2 million cases per year (Philips *et al*., 2010).Children aged under 5 years residing in resource-rich countries, NoVs cause ~900 000 episodes of gastroenteritis necessitating a clinic visit, compared with resource poor countries in which NoVs may cause more than one million hospitalizations and up to 200 000 deaths each year(Patel *et al*., 2008). As enteric viruses, the NoVs are spread via the feces or vomitus of infected individuals. Norovirus illness is contracted through contaminated food and water and direct person-to-person transmission. Contamination often arises during the handling and preparation of foods (Jiang et al 1990).*Norovirus*, belonging to the family of Caliciviridae,NoVs are non-enveloped viruses with a single-stranded, positive-sense, polyadenylated RNA genome of about 7500 nucleotides (nt) in length. Three overlapping ORFs encode the nonstructural (ORF1) and structural (ORF2 and ORF3) viral Proteins (Green *et al*., 2000). NoV in nature is genetically and antigentically highly diverse(Kageyama*et al*.,2004). NoV is tentatively divided into five genogroups (GI to GV) Most NoVs infecting humans belong to genogroups GI and GII. GI genogroup is further subdivided into at least eight genotypes and GII genogroup into 17 genotypes. Is a highly contagious virus and has been found to be one of the most important causes of nonbacterial acute gastroenteritis in all ages in developing as well as in developed countries (Jiang *et al*., 1990;Lopmann *et al*., 2004). While most of the outbreaks are known to have a seasonal pattern, sporadic cases of disease throughout the year are described(Piang*et al*.,2000) .Specific diagnosis of Norovirus is routinely made by

polymerase chain reaction (PCR) assays or real-time PCR assays, which give results within a few hours. These assays are very sensitive and can detect as few as 10 virus particles(Marshal,and Bruggink2006).

## Material and Methods

### Sample collection

Stool samples were collected from children under 5 years with clinical symptoms of non-bacterial or parasitic acute gastroenteritis: nausea, vomiting and/or three or more loose stools in 24 hours during the acute phase of the infection in sterile plastic water prof container labeled with patient name ,patient number ,hospital ,and date of collection Figure 1 ; Samples were transported to the laboratory on ice in sealed bag stored at +4ºC in refrigerator until processing. After examination, samples were stored at
-20ºC.( Hansman**,** 2007;Anbazhagi *et al*., 2011).

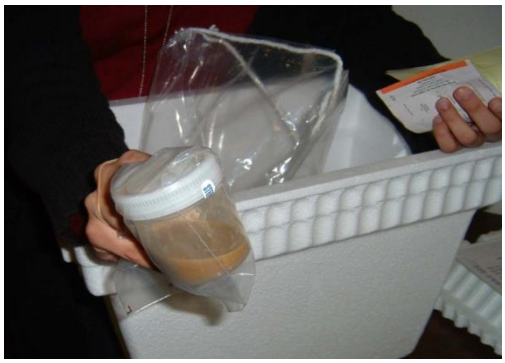

**Figure 1. Sample collection**

### RNA Extraction

For viral RNA extraction 30% (w/v) stool suspensions were made in phosphate-buffered saline (PBS;7.2pH).Extractions were performed using the QIAamp1Viral RNA Mini kit (Qiagen, Germany) according to the manufacturer'sinstructions.

### Real-time quantitative reverse transcription-PCR (RT-PCR )(Lazaro,2010).

Viral RNA was extracted from 140 µL of supernatant with a QIAamp viral RNA mini kit (Qiagen, Germany). Real-time quantitative RT-PCR was performed using Go Tag 1 step RT -qPCR reaction kit from (Promega). Three sets of specific primers, QNINF4, NV1LCR,and NVGG1P probe were used to detect NV GI and QNIF2, COG2R and QNIFS probe were used to detect NVGII, respectively with using IACP(Applied biosystem UK). Real-time RT-PCR was performed with an ABI Prism 7500 sequence detector (Applied Biosystem) under the following conditions: (1) Revers transcription ≥ 37°C for 15 min, (2) Hot start activation 95°C for 10 min,(3)steps q PCR 40 cycles of amplification with denaturation at 95 for 10 sec, annealing at 60°C for 30 sec and extension at 72°C for 30 sec.

### ROTA-VIRUS LATEX TEST KIT

It is a rapid latex agglutination assay for the detection of Rotavirus in fecal samples.Stool suspensions were prepare a 10% suspension of the fecal sample by adding 0.1ml/0.1g of sample to 1ml of extraction buffer in a screw capped vial. Mix well. Stand at room temperature for 2 minutes ,then Examined according to the manufacturer's instructions.

## Results

**Table 1.The incidence of human Norovirus and Rotavirus in five age groups (%) from 252 samples of different gender**.

| Age groups year | NV GI Positive sample | | NV GII positive sample | | Rotavirus positive sample | | Negative sample | |
|---|---|---|---|---|---|---|---|---|
| | ♂(%) | ♀(%) | ♂(%) | ♀(%) | ♂(%) | ♀(%) | ♂(%) | ♀(%) |
| 0-≤1 | 9 (69.23) | 7 (70.0) | 22 (70.96) | 15 (55.56) | 21 (67.74) | 20 (64.52) | 36 (59.01) | 26 (54.16) |
| 1-≤2 | 4 (30.76) | 2 (20.0) | 2 (6.45) | 7 (25.93) | 6 (19.36) | 9 (29.03) | 10 (16.39) | 10 (20.01) |
| 2-≤3 | 0 (0.00) | 1 (10.0) | 3 (9.67) | 4 (14.81) | 0 (0.00) | 1 (3.23) | 7 (11.47) | 2 (4.16) |
| 3-≤4 | 0 (0.00) | 0 (0.00) | 1 (3.23) | 1 (3.70) | 1 (3.23) | 1 (3.23) | 2 (3.27) | 6 (12.5) |
| 4-≤5 | 0 (0.00) | 0 (0.00) | 3 (9.67) | 0 (0.00) | 3 (9.68) | 0 (0.00) | 8 (13.11) | 4 (8.33) |
| Chi-square | 11.54 ** | 10.94 ** | 10.78 ** | 8.63 ** | 10.03 ** | 9.64 ** | 8.36 ** | 8.12 ** |
| Total | 13 (5.1) | 10 (3.96) | 31 (12.30) | 27 (10.71) | 31 (12.3) | 31 (12.3) | 61 (24.2) | 48 (19.04) |
| 252 | 23 | | 58 | | 62 | | 109 | |

** (P<0.01).

**Table 2. The number or Rotavirus and Norovirus positive samples and co-infection between May 2012 and May 2013 in Baghdad (%).**

| Month | No of Rotavirus Positive Sample (%) | No of Norovirus Positive Sample (%) | No of Norovirus Rotavirus co-infection (%) | No of negative cases (%) | No of total cases | Chi-square value |
|---|---|---|---|---|---|---|
| May | 2 (10.5) | 2 (10.5) | 0 (0) | 15 (78.9) | 19 | 4.051 * |
| June | 5 (20) | 12 (50) | 0 (0) | 8 (33.3) | 24 | 10.54 ** |
| July | 9 (45) | 4 (40) | 0 (0) | 8 (40) | 20 | 10.82 ** |
| August | 6 (28.57) | 14 (66.6) | 2 (9.5) | 4 (19) | 21 | 9.46 ** |
| September | 5 (23.8) | 1 (4.8) | 0 (0) | 15 (71.4) | 21 | 6.84 ** |
| October | 7 (30.4) | 5 (21) | 2 (8.7) | 13 (56.5) | 23 | 7.72 ** |
| November | 3 (13.6) | 3 (13.6) | 0 (0) | 17 (77.3) | 22 | 3.68 * |
| December | 4 (20) | 7 (35) | 0 (0) | 9 (45) | 20 | 7.91 ** |
| January | 7 (31.8) | 9 (40.9) | 4 (17.4) | 12 (52.2) | 23 | 8.52 ** |
| February | 5 (26.3) | 5 (26.3) | 0 (0) | 10 (52) | 19 | 7.39 ** |
| March | 4 (20) | 11 (55) | 1 (5) | 8 (40) | 20 | 9.43 ** |
| April | 5 (25) | 8 (40) | 2 (10) | 10 (50) | 20 | 9.18 ** |
| Total | 62 (24.60) | 81 (32.14) | 11 (4.36) | 129 (51.19) | 252 | |

**** (P<0.01).**

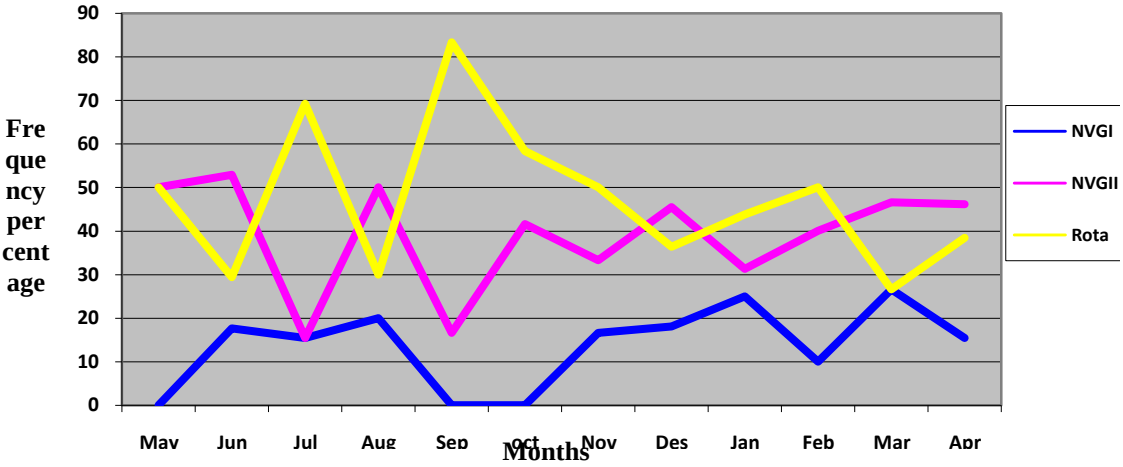


**Figure 2.The Frequency percentage of *Norovirus GI* ,*Norovirus GII* ,*and Rotavirus* positive samples between May 2012 and May 2013**

**Table 3 .The number of *Norovirus GI* ,*Norovirus GII* ,and Co-infection positive samples between May 2012 and May 2013 (%).**

| Month | No of Norovirus G1 positive Sample(%) | No of Norovirus G2 positive Sample(%) | No of Co-infection NVG1& NVG2(%) | No of total cases | Chi-square value |
|---|---|---|---|---|---|
| May | 0 (0) | 2 (10.5) | 0 (0) | 19 | 12.56 ** |
| June | 3 (12.5) | 9 (37.5) | 1 (4.2) | 24 | 8.63 ** |
| July | 2 (10) | 2 (10) | 1 (50) | 20 | 9.12 ** |
| August | 4 (19) | 10 (47.6) | 2 (9.5) | 21 | 9.05 ** |
| September | 0 (0) | 1 (4.8) | 0 (0) | 21 | 11.38 ** |
| October | 0 (0) | 5 (21.7). | 0 (0) | 23 | 9.62 ** |
| November | 1 (4.5) | 2 (9.1) | 0 (0) | 22 | 12.85 ** |
| December | 2 (10) | 5 (25) | 0 (0) | 20 | 9.63 ** |
| January | 4 (17.4) | 5 (21.7) | 1 (4.3) | 23 | 9.24 ** |
| February | 1 (5.3) | 4 (21) | 1 (5.2) | 19 | 10.63 ** |
| March | 4 (20) | 7 (35) | 2 (10) | 20 | 9.21 ** |
| April | 2 (10) | 6 (30) | 1 (5) | 20 | 10.78 ** |
| Total | 23 (9.13) | 58 (23) | 9 (3.57) | 252 | 10.32 ** |

** (P<0.01).

**Table 4. The Gastrointestinal symptoms in Norovirus in 81 positive cases**

| Gastrointestinal symptoms | Percentage (%) |
|---|---|
| Abdominal pain | 83.93 |
| Vomiting | 91.07 |
| Diarrhea | 98.21 |
| Severe dehydration | 1.80 |
| Some dehydration | 33.90 |
| No dehydration | 46.42 |
| Nausea | 51.80 |
| Normal temperature | 80.30 |
| Mild temperature | 16.07 |
| fever | 3.57 |
| Chi-square value | 13.287 ** |

** (P<0.01).

**Table 5.The co-infection and mono-infection in relation to duration ,vomiting and diarrhea episodes among 143 Rotavirus and Norovirus positive cases**

| Disease Manifestation | Norovirus Mono-infection | Rotavirus Mono-infection | NVGI& NVGII mix-infection | NV &Rotavirus Mix-infection |
|---|---|---|---|---|
| **Duration** | | | | |
| <4 day | 50 (81.96) | 20 (39.21) | 6 (66.6) | 1 (9.09) |
| 4-7 day | 10 (16.39) | 27 (52.94) | 2 (22.2) | 4 (36.36) |
| <7 day | 1 (1.63) | 4 (7.84) | 1 (11.1) | 6 (54.54) |
| Chi-square | 13.58 ** | 9.43 ** | 10.45 ** | 9.27 ** |
| **Vomiting episode/day** | | | | |
| <3/d | 22 (36.06) | 14 (27.45) | 2 (22.22) | 1 (9.09) |
| 3-7/d | 31 (50.81) | 22 (43.13) | 2 (22.22) | 3 (27.27) |
| >7/d | 8 (13.11) | 15 (29.41) | 5 (55.56) | 7 (63.63) |
| Chi-square | 9.63 ** | 11.49 ** | 9.54 ** | 11.43 ** |
| **Diarrhea episode/day** | | | | |
| <3/d | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| 3-7/d | 39 (63.93) | 35 (68.62) | 4 (44.44) | 4 (36.36) |
| >7/d | 22 (36.06) | 16 (31.37) | 5 (55.55) | 7 (63.63) |
| Chi-square | 11.67 ** | 11.75 ** | 10.43 ** | 11.94 ** |
| Total | 61 | 51 | 9 | 11 |

** (P<0.01).

## Discussion

Through this study, which investigated children under five years with acute gastroenteritis(AGE) attending hospitals in Baghdad .A number of 252 samples were collected and information was extracted from patient using Diarrhea case report form .All samples were Examined using RT-PCR (the genomic region utilized to detect and genotype HuCV by RT-PCR codify to the polymerase gene (RdRp)),which is relatively conserved in both genera. Table (3.1) shows that the overall number of males was higher than female children in AGE by 136 (53.9%) males and 116 (46.03%) females. Also the result revealed a higher incidence of Norovirus infection in males compared to females. As we have noted the infection with genogroup NVGI was affecting 13 males (5.1%) of AGE and effecting only 10 females (3.96%) of AGE, and the occurrence of infection with genogroup NVGII was effecting 31 males (12.30%) of AGE in comparison to only 27 females (10.71%) of AGE .While the study found through equal readiness of Rotavirus infection between males and females and by 31(12.30%) cases for both of them with a high significant value as shown in Table (1) . The recurrence Norovirus infection under one year and in males were more than in females. The result showed increase in Norovirus infection for the same age group and superiority over Rotavirus. The lower incidence in older ages for both viruses, is probably due to their past infection in early childhood which might earn immunity against infection in the future and also using *Rotavirus* vaccine.

These results were expected by Gómez-Santiago *et al*. (2012) in Brazil and with Mandell*et al*. (2010) which refers to the age group most affected with HuCV infection (7-18 months) corresponded to the group most commonly affected by *Rotavirus*, with a median age of 12 months.Throughout the study appeared emergence of NVG infection during all months throughout the different seasons , (Table 2). This correspond with Beek*et al* .(2013) who get the same pattern throughout the year.Also this study showed a high incidence of NVG during August 66.6% ,March (55% ),June (50%) and January (40.9%) ,and dominated NV GGII genogroup on NVGGI genogroup . These results was correspond with Chan and Chan ( 2013) in Hong Kong, throughout 2012, refers to *Norovirus* activity peaked in August and subsided in October, in addition to Glass and Parashar (2009) also observed a higher incidence in the summer months compared to the winter months .Our results was not correspond with studies in many of countries indicate that epidemics of NVG outbreaks tend to occur each year with a peak in colder months in northern hemisphere countries as observed by Goller and Dimiriadis (2004) ,and a peak in warmer months in southern hemisphere countries, such as Australia as mentioned by Lazaro (2010) and Bruggink and marshal( 2009).Also Gómez-Santiago *et al*. (2012 ) was refers to the distribution of human caliciviruses (HuCV) infection occurred mainly during two periods: November-December, and April-June, in contrast with Brazil, where infection has been reported along the entire year without a seasonal pattern (Soares*et al*., 2007).But in Chennai city of southern India, Anbazhagi*et al* (2011) referred in their study to that prominent numbers of NoV infection was observed on April 2006 followed by January 2006, whereas Norovirus is generally referred to as "winter-vomiting disease", In comparison to this study(in Iraq) a contrary picture observed as we see that is high in the summer season especially during August .On the other hand Rotavirus infection spread throughout the year and a significant increase in the July (45%) in the summer months and in January (31%) in the winter months as can be noted in (Figure 2).The results demonstrates a high prevalence of NoV infections in neonates.Mixed infections and their distribution according to monthsare shown in Tables 2,and 3 . *Norovirus* and *Rotavirus* co- infection was seen in 11 cases presented 17.4% , 10% ,9.5% ,8.7% ,and 5% of positive cases in ,January , April ,August , October ,and March respectively .This indicates that co-infection with *Rotavirus* may result in significantly more gastrointestinal disease in this age group. NVGI, and NVGII co-infection were found in 9 cases presented 3.5% of all collected samples and appear through highly prevalence months in January ,February ,March ,April, June ,July, and August. Mixed infections were predominantly detected in the under 1years age group and this is consistent with observations seen by Roman*et al*.( 2003) who explains that virus–virus co-infections were significantly more frequent in 7-18 months age group. Also the majority of these co-infection were combinations of *Rotavirus* with other viruses (*Astrovirus* ,*Adenovirus* or *Calicivirus* ) . Detection of more than two infectious agents was infrequent. As it has been noted in this study that mixed infections are less frequent than mono-infections but with more sever and prolong duration time , and this correspond with the result done by Prats*et al*. (1997) in their study In Barcelona, Spain The Statistical comparison of single virus infection with double infections is complex because most of the co-infection groups are small. (Table 5), it has been shown that the most important exhibitors of patients were vomiting, and diarrhea , a high proportion 91.01%, 98.21% respectively .A high rate of patient 83.93% were suffering from abdominal pain and moderate nausea 51.8%. Also severe dehydration and fever were occurred only in 1.80% and 3.57% of patients on the other hand most patient have normal body temperature and very low

rate of dehydration in children and these finding consistent with Graham *et al* .(1994) results who explained that Norovirus can be detected in infected individuals with no major symptoms.

The symptoms between the mono-infection groups and mixed infection group were compared (Table 5),and found that rotavirus caused a higher frequency and longer duration of vomiting than did Norovirus.This can explained *Norovirus* RNA excretion can continue for long periods for up to 44.5 days following onset of symptoms in a naturally infected individual as mentioned by Menon*et al*. (2010). This may explain the causes behind the mixed infection with Rotavirus in asymptomatic patients in this study.

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