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# Relative Frequency of Norovirus Infection in Children Suffering From Gastroenteritis and Referred to Aboozar Hospital, Ahvaz, Iran

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**ABSTRACT**

*Background:* Noroviruses belong to the *Norovirus* genus in the *Caliciviridae* family. Noroviruses are the most common causes of gastroenteritis and have a great impact on public health. They have been identified as a common cause of acute gastroenteritis in children.
*Objectives:* The aim of this study was to determine the prevalence of Norovirus in children suffering from gastroenteritis.
*Patients and Methods:* Fecal samples (n = 143) were collected from children under 5 years of age who were suffering from gastroenteritis. All the children were referred to Ahvaz Aboozar Hospital, located in southwestern Iran. Norovirus RNA was extracted by Trizol, and RNA was detected using nested reverse transcriptase polymerase chain reaction (nested-RT-PCR).
*Results:* Norovirus infection was detected in 9 of the 143 collected samples (6.3%). All positive samples belonged to genogroup II. Five positive samples were obtained from male patients and 4 were obtained from female patients. Most of the positive cases were from patients between 3 and 5 yars of age (n = 5, 56%). There was no relationship between gender and virus prevalence. The rate of infection peaked in winter (n =6, 66.9%), and we did not detect any positive cases in summer.
*Conclusions:* The prevalence of this virus in Ahvaz is similar to that reported by other researchers. Because this virus is transmitted by contaminated food or water, we recommend adult education and improved personal hygiene to reduce the incidence of Norovirus infection in children. This study improves our epidemiological knowledge of the prevalence of this virus in Ahvaz and Iran.



*Implication for health policy/practice/research/medical education:*
This study improves our knowledge about the significance of noroviral gastroenteritis in clinics.

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## 1. Background

Noroviruses belong to the *Norovirus* genus in the *Caliciviridae* family (1). The virion contains a single positive-strand RNA genome with icosahedral symmetry. Based on the genetic sequence of viral RNA-dependent RNA polymerase and the capsid proteins, noroviruses are divided into 5 genogroups GI-GV (2). Traditionally, noroviruses were detected using electron microscopy. The

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limitation of this method was the low loud of viruses, because EM visualization requires $10^6$ viral particles per gram of fecal sample (3).

Noroviruses are the most common causes of gastroenteritis, and they have a great impact on public health (4). They have been identified as a common cause of acute gastroenteritis in children (5). Patients infected with Norwalk-like viruses (NV) present with the following signs and symptoms : nausea (79%), vomiting (69%), diarrhea (66%), abdominal cramps (30%), headache (22%), fever (subjective; 37%), chills (32%), myalgia (26%), and sore throat (18%). Bloody stools have not been reported until recently (6). Attempts to develop an inclusive serological test for detection of all noroviruses have not been successful because of the antigenic diversity of these viruses (7).

## 2. Objectives

Since the total genome of noroviruses has been sequenced, detection of these viruses can be performed by sensitive molecular methods such as nested RT-PCR. This method is between 10 and 1000 times more sensitive than conventional RT-PCR (8). Therefore, we used nested RT-PCR to detect noroviruses in fecal specimens collected from children less than 5 years of age who were referred to Ahvaz Aboozar Hospital in Iran. This information will improve our epidemiological knowledge about the prevalence of this virus in Ahvaz and Iran.

## 3. Patients and Methods

Fecal specimens were collected from children under 5 years of age who had gastroenteritis and were referred to Aboozar Hospital in Ahvaz between 2008 and 2009. Samples from 143 patients were collected and stored at -80 °C. Samples from patients with bacterial infections or parasite infestations were excluded from the study. We tested all the samples for noroviral infection by using nested RT-PCR.

### *3.1. RT-PCR*

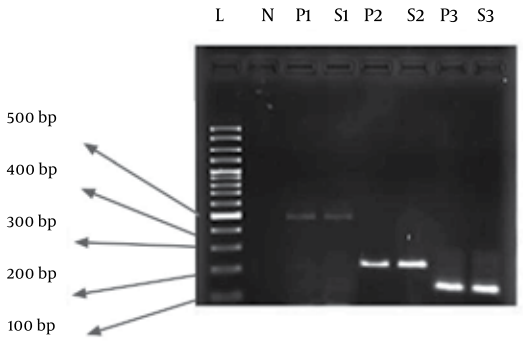
The RNA of viruses was extracted by Trizol (Roche Germany). The extracted RNA was denatured by incubation at 60 °C for 5 min and then incubated on ice for 2 min. We used a Fermentas RT-PCR kit (Lithuania) to construct cDNA from 9 μl of extracted RNA, according to the manufacturer's instructions. Five microliters of the cDNA was used as a template for PCR with the following primers: NV 35 (5′-CTTGTTGGTTTGAGGCCATAT-3′) and NV 36 (5′-ATAAAAGTTGGCATGAACA-3′) (9). These primers amplify 470 bp from open reading frame (ORF) 1a that encodes RNA polymerase. We used the following PCR conditions: 35 cycles of 1 min at 94°C, 1 min at 49°C, and 1 min at 72°C, followed by a final extension of 6 min at 72°C. The products of the first round of PCR were subjected to electrophoresis in 3% agarose gel, stained by ethidium bromide, and visualized under a UV Transilluminator (Vilber Lourmat, France). We took a 1-μl subsample of each PCR product of the first round of PCR, including those that were both positive and negative for Norovirus. Using NV 51 (5′-GTTGACACAATCTCATCATC-3′) and NV 3 (5′-GCACCATCTGAGATGGATGT-3′) primers, we amplified 206 bp under the following PCR conditions: 30 cycles of 1 min at 94°C, 1 min at 42°C, and 1 min at 72°C, followed by a final extension of 6 min at 72°C. The PCR products were loaded on a 3% agarose gel, stained, and visualized under a UV Transilluminator (10).

To determine whether samples contained Norovirus from genogroup I or genogroup II, 2 pairs of specific genogroup primers were used in a PCR reaction. For group 1, these were CAP A (5′-GGC (A/T) GTT CCC ACA GGC TT-3′) and CAP B (5′-TAT GTT GAC CCT GAT AC-3′). For group 2, the specific primers were NVP 110 (5′-AC(A/T/G) AT(C/T)TCATCATCACCATA-3′) and SR 46 (5′-TGGAATTCCATCGCCCACTGG-3′) (11). These primers amplify 178 bp from ORF 2 that encodes a capsid protein in genogroup I and 119 bp from ORF 1a that encodes RNA polymerase in genogroup II. The PCR conditions were as follows: 30 cycles of 30 sec at 94°C, 30 sec at 50°C, and 45 sec at 72°C followed by a final extension of 5 min at 72°C (*Figure 1*).

## 4. Results

After the second round of PCR, Norovirus was detected in 9 of 143 (6.3%) fecal samples. All the positive samples belonged to genogroup II and there were no positive Norovirus genogroup I samples. Five positive samples were from male patients and 4 were from female patients. There was no relationship between gender and the prevalence of the virus. The peak infection rate was observed in winter (66.9% of positive cases), and we did not detect any positive cases during the summer.

**Figure 1.** Representative Agarose Gel Electrophoresis of PCR Products From A Positive Norovirus Sample.




P lanes show results from a positive control and S lanes are the results from a field sample. The number 1 indicates results of first-round PCR, the number 2 indicates the results of nested PCR, and the number 3 indicates genogroup test results. A 100-bp DNA ladder was loaded into the L lane.

## 5. Discussion

Acute gastroenteritis is one of the most common diseases in the human population (12). Annually, up to 700

million cases of this disease occur in children under 5 years of age. Usually, gastroenteritis causes mild diarrhea. However, patients may show more severe symptoms ranging from relatively mild upper gastroenteritis symptoms, such as nausea and vomiting, to severe symptoms, such as profuse diarrhea that leads to dehydration or death (13). Human caliciviruses, especially noroviruses, are important causes of nonbacterial gastroenteritis. They are the most important pathogen transmitted through contaminated food (14).

According to studies from all over the world, the prevalence of this virus is between 6%-19% (15). For example, O'Neill et al found that the prevalence of this virus was 6.7% in Ireland (16). In Germany, Rohayem et al determined that the prevalence of Norovirus was 6.6% during one study interval and 9.5% during a second study interval (17). In our study, the prevalence of Norovirus was 6.3%, very similar to the prevalence reported by these authors. Children under 5 years of age show higher rates of infection with this virus than people in other age groups. Among these children, the peak infection rate occurs between the ages of 3 and 5 years. In a study performed in Bangladesh, researchers found Norovirus antibodies in 43% of the children between 2 and 49 months of age. The authors of this study found that only 7% of 2–7-month-old children harbored Norovirus antibodies, but 100% of 4-year-old children had them (18). We found that 6.3% of the fecal samples from children under 5 years of age contained noroviruses. Additionally, 55.6% of these samples were from children between 3 and 5 years of age. Children under 1 year of age had the lowest prevalence of Norovirus infections (22.2%). Our results are in concordance with studies undertaken in other areas of the world. Ike *et al*. reported that out of the 148 fecal samples they tested, 95% were positive for noroviruses belonging to genogroup II (19). This report matches our results and those of similar studies showing that genogroup II is the dominant group all over the world (20, 21). Human caliciviruses, especially Noroviruses, are important causes of nonbacterial gastroenteritis. The prevalence of this virus in Ahvaz is similar to that in other studies. Because this virus is transmitted through contaminated food or water, drinking-water sanitation greatly reduces the incidence of Norovirus gastroenteritis. In addition, this study improves our epidemiological knowledge about the prevalence of this virus in Ahvaz and Iran.

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