Incidence of Picornaviruses among Children with Respiratory Tract Infections Attending Ain Shams Pediatric University Hospital, Egypt


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Abstract

Background: Respiratory infections represent a major public health problem. Human rhinoviruses (HRVs) and enteroviruses (HEVs) are prominent causes of respiratory diseases. Reverse transcription-PCR (RT-PCR) is a highly sensitive technique for the detection of nucleic acid sequences from RNA viruses in clinical specimens. This work was designed to measure the incidence of picornaviruses (HRVs and HEVs) in children with acute respiratory tract infections (ARTI) attending or admitted to Ain Shams Pediatrics University Hospital between January 2015 and March 2016.

Methods: One hundred and sixty-one children were enrolled in this study. Upper respiratory tract and stool samples were collected from all children and HRVs and HEVs were detected by pan-picornavirus real time RT-PCR and virus isolation in tissue culture.

Results: HRV was detected in 54 respiratory tract samples (33.5%) by RT-PCR, while by culture only one HRV (0.6%) was detected. HEV was detected in 18 stool samples (11.2%) by RT-PCR, while by culture HEV was detected in only four (2.5%) samples. Sensitivity, specificity, positive predictive value and negative predictive value of viral culture considering RT-PCR as the gold standard were 7.46%, 100%, 100% and 57.05% respectively. The most frequently detected species of picornaviruses were HRV-C, followed by HRV-A, then HEV-A and HEV-C.

Conclusion: Picornaviruses were responsible for a high percentage of ARTI among the children under investigation. RT-PCR was superior in rapidity and improvement of the laboratory diagnoses of ARTI, compared with virus isolation and is thus recommended as the primary diagnostic tool.

Keywords: Picornaviruses; Pediatric Patients; Acute Respiratory Tract Infections; RT-PCR; Virus Isolation

Abbreviations

HRVs: Human Rhinoviruses; HEVs: Human Enteroviruses; RT-PCR: Reverse Transcription-PCR; ARTI: Acute Respiratory Tract Infections; URTI: Upper Respiratory Tract Infections; LRTI: Lower Respiratory Tract Infections; CPE: Cytopathic Effects
Introduction

Respiratory infections represent a major public health problem because of their high incidence and ease of spread in the community [1]. Acute respiratory tract infections (ARTIs) are associated with significant morbidity and are the most common reason for outpatient visits and hospitalizations among young children [2]. Human rhinoviruses (HRVs) and enteroviruses (HEVs) are prominent causes of respiratory diseases [3].

HRV infection often results in mild upper respiratory disease like the common cold, but may also cause more serious disease by exacerbating asthma or other pre-existing respiratory disorders. In contrast, HEVs infect primarily the gastrointestinal tract and can spread to other sites, but some HEVs display specific tropism for the respiratory tract [4]. HRVs circulate throughout the year, with occurrence typically peaking in spring and autumn in the temperate regions [5].

For laboratory diagnosis of picornavirus (HRV and HEV) infections, collection of nasopharyngeal aspirates is preferred for upper respiratory tract infections (URTI) while bronchial or tracheal aspirate samples are preferred for lower respiratory tract infections (LRTI) [6]. HRV can be detected in approximately 10% of stool samples from hospitalized patients, predominantly from children under the age of 2 years [7]. Laboratory testing is performed by molecular methods such as real time RT-PCR or by routine virus isolation in cell culture. HEV and HRV share high similarity in the 5′ non-translated region (5′ NTR) [8] and can be detected by a generic RT-PCR assay targeting this 5′ NTR of the viral genome [9].

Aim of the Study

The aim of this study was to measure the incidence of picornavirus infections (HEV and HRV) in children with acute respiratory tract infections attending Ain Shams Pediatrics University Hospital in Cairo, Egypt.

Patients and Methods

Samples were collected from 161 children admitted to or attending Ain Shams Pediatric University Hospital for ARTI during the period from January 2015 to March 2016. The study was approved by the Hospital Ethics Committee of Ain Shams University Hospitals and informed consent was taken from the children’ parents and an authorized person in either the clinic or the ward. The age of the children ranged from 1 - 10 years. Inclusion criteria were the following: age from one year to ten years, hospitalized for acute expiratory wheezing. Exclusion criteria were the following: chronic diseases as cystic fibrosis, bronchopulmonary dysplasia, congenital heart disease and chronic allergic rhinitis. A nasal swab or a throat swab or a nasopharyngeal aspirate and a stool sample were collected from each child. Picornaviruses (HEVs and HRVs) were detected using a pan-picornavirus real time RT-PCR assay [10] and pan-HRV [11] and pan-HEV real time RT-PCR assays [12]. In addition, samples were inoculated in tissue culture plates for virus isolation, after which virus replication was monitored by scoring cytopathic effects (CPE) for up to 2 weeks. A subset of the picornavirus positive specimens (n = 13) was used for sequence analysis of the (partial) virus genome.

RNA extraction

Extraction of viral RNA from respiratory specimens and stool was carried out using a commercial RNA isolation procedure (Qiagen, viral RNA extraction kit (Germany), according to the manufacturer instruction.

Real time RT-PCR

One step real time RT-PCR was performed using the quantitect probe RT-PCR master mix (Qiagen Germany) in a total volume of 25 μl per sample. Each tube contained 12.5 μl of the ready master mix plus 1 μl of each primer and probe completed to 20 μl with PCR grade water and 5 μl of each sample. For the pan-picornavirus real time RT-PCR the following primers and probe were used: HRV F1 (5’GGT GTG AAG ASY CVC RTG TGC T 3’), HRV R1 (5’GCT STR GGK TTW AGG TTA GCC 3’) and HRV TMI (Fam 5 ‘TGA GTC CTC CGT CCC CTG AAT G 3’hq). For detection of HEVs among pan-picornavirus positive samples the following HEV specific primers and probe were used: En-
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Enterovirus forward (5’CCC TGA ATG CGG CTA ATC C3’), Enterovirus Reverse (5’ATT GTC ACC ATA AGC AGC CA 3’) and Enterovirus TM (Fam 5’AAC CGA CTA CTT TGG GTG TTC C3’ TAMARA). For detection of HRVs, the following HRV specific primers and probe were used: RHINO F (5’AGC CTG CGT GGC KGC C 3’), RHINO R (5’GAA ACA CGG ACA CCC AAA GTA GT 3’) and probe (Fam 5’SCT CGG CCC CTG AAT GYG GCT AA BHQ). Real time RT-PCR was performed using a One Step plus real time machine (Applied Biosystem USA), using the following reaction conditions: initial reverse transcriptase step at 50ºC for 30 minutes, followed by hot start enzyme activation at 95ºC for 15 minutes, followed by 40 cycles of 95ºC for 30 seconds, 60ºC for 1 minute. All runs contained positive, negative, and no template controls. RT-PCR positive samples were identified automatically, when the fluorescence signal exceeded the threshold level determined by the machine.

Virus isolation in tissue culture

Sample processing

The nasal swabs or throat swabs or nasopharyngeal aspirates and stool samples were processed immediately or frozen at -80 ºC until processed. The swabs were removed from the transport tube and the contents were broken up with sterile glass beads by vortexing. Stool samples were mixed as 10% in viral transport medium and vortexed. All samples were centrifuged at 4000 rpm for 20 minutes. Supernatant from each sample (200 ul/cell line) was used for cell culture inoculation after filtration through 0.22 μm Millipore syringe filter.

Three cell lines - Vero (African green Monkey kidney cells), RD (Rhabdomyosarcoma cell) and A549 (Human lung carcinoma) cells - were used for virus isolation. The rationale for using the three cell lines was to cover as much as we can of the picornaviruses that can be grown from samples. Note that although Human fetal embryonic lung fibroblast cell lines and HeLa cells are known to be highly permissive for picornaviruses, we couldn't use them because they were non-available at the time of the study from local supplier.

The cells were grown in tissue culture flasks (Corning USA) using Iscoves modified Dulbecco’s medium (Sigma company) supplemented with 10% fetal bovine serum (Gibco USA), penicillin, streptomycin and fungizone. Flasks were incubated at 37ºC until the cell monolayer was semi-confluent. Growth medium was removed and 0.2 ml of processed sample was inoculated in the three cell lines, and incubated at 37ºC for one hour. Subsequently, the incubation maintenance medium (the same as growth medium but with fetal bovine serum concentration at 2%) was added and flasks were incubated for 2 weeks. Cell monolayers were observed daily under an inverted microscope for the detection of cytopathogenic effects (CPE). Maintenance medium for each flask was changed every other day. CPE were in the form of rounding of cells and cell detachment. When CPE were detected, passage to a fresh culture flask was done to confirm the CPE and to exclude toxic effects of the sample. Furthermore, identification of the growth as picorna viruses were done by indirect immunofluorescence assay using monoclonal antibodies reactive for most enteroviruses (Dako cat no: M 7064 Clone 5-D 8/1) and extraction of RNA and testing by real time PCR to detect entero and Rhino. Picornavirus identification from flasks with CPE was done using the same real time RT-PCR used for the direct specimens.

Sequencing

The 5’UTR and VP4/VP2 regions (approximately 290 and 542 nucleotides, respectively) of selected HRV and HEV positive samples (n = 13) were amplified by RT-PCR. Initial PCR for VP4/VP2 was done using a forward primer (5’-CGG CCC CTG AAT GYG GCT AA-3’) and reverse primer (5’-TCN GGN ARY TTC CAC CAN CC -3’) and semi-nested PCR was performed with a second forward primer (5’-CTA CTT TGG GTG TCC GTG TTT C-3’) and the same reverse primer as in the first reaction [13,14]. Initial PCR for the 5’UTR was done using a primer (5’-CAA GCA CTT CTG TTT CCC GGC-3’) and reverse primer (5’-GAA ACA CGG ACA CCC AAA GTA GT-3’) and semi-nested PCR was performed with the same forward primer as in the first reaction and a second reverse primer (5’-CAT TCA GGG GCC GGA GGA-3’) [15]. The PCR products were purified from agarose gels and sequenced using Big Dye Terminator v 3.1 (Applied biosystems).

Statistical analyses

Data were analyzed using a personal computer Statistical software package version 5 (Stat Soft Inc. USA). Quantitative data were statistically represented in terms of minimum, maximum, mean, and standard deviation (SD). Comparison between two groups was done us-
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The results of this study indicate that the incidence of Picornaviruses among children with respiratory tract infections at Ain Shams Pediatric University Hospital, Egypt, was 33.5% for HRV and 11.2% for HEV. The age range of the children enrolled in the study was 1 - 10 years (mean ± SD 5.48 ± 2.09), with 73 (45.3%) males and 88 (54.7%) females. Most of the children (140, 86.96%) suffered from URTI, while 21 (13.4%) were affected by LRTI. One hundred and three children (64%) presented with acute bronchitis, 37 (23%) with other URTI, 15 (9.3%) with atypical pneumonia, and 6 (3.7%) with pneumonia.

The RT-PCR assays had a higher detection rate than conventional viral culture for HRV and HEV. HRV was detected by RT-PCR in 54 of 161 (33.5%) respiratory samples but only in 1 (0.6%) by virus culture. HEV was detected by RT-PCR in 18 of 161 stool samples (11.2%) and in 4 of 161 samples by virus culture (2.5%) (Table 1). The sensitivity, specificity, positive predictive value, and negative predictive value of viral culture when compared to RT-PCR were 7.46%, 100%, 100%, and 57.05% respectively (Table 2).

The pattern of seasonal variation of picornavirus infections, starting from January 2015 until March 2016, is shown in figure 1. Both HRV and HEV peaked in May 2015, although both were present throughout the study period.

**Results**

The age of the children enrolled in this study ranged from 1 - 10 years (mean ± SD 5.48 ± 2.09). Of these children, 73 (45.3%) were males and 88 (54.7%) were females. Most of the children enrolled in this study (140, 86.96%) suffered from URTI while the remaining 21 children (13.4%) were affected by LRTI. One hundred and three children (64%) presented with acute bronchitis, 37 (23%) presented with other URTI, 15 (9.3%) presented with atypical pneumonia and 6 (3.7%) presented with pneumonia.

The RT-PCR assays had a higher detection rate than conventional viral culture for HRV and HEV. HRV was detected by RT-PCR in 54 of 161 (33.5%) of the respiratory samples collected from the children but only in 1 child (0.6%) by virus culture. HEV was not detected in these respiratory samples. In contrast, HEV was detected by RT-PCR in 18 of 161 stool samples collected from these children (11.2%) and in 4 of 161 samples by virus culture (2.5%) (Table 1). Table 2 shows that the sensitivity, specificity, positive predictive value and negative predictive value of viral culture when compared to RT-PCR were 7.46%, 100%, 100% and 57.05% respectively.

**Detection method**  | **Specimens**  | **Virus**  | **N** | **%**
---|---|---|---|---
**PCR**  | Respiratory specimens  | Negative  | 106  | 65.8
        | Rhino virus  | 54  | 33.5
        | Total  | 161  | 100.0
        | Stool specimens  | Negative  | 143  | 88.8
        | Enterovirus  | 18  | 11.2
        | Total  | 161  | 100.0
**Culture**  | Respiratory specimens  | Negative  | 160  | 99.4
        | Rhino virus  | 1  | 0.6
        | Total  | 161  | 100.0
        | Stool specimens  | Negative  | 157  | 97.5
        | Enterovirus  | 4  | 2.5
        | Total  | 161  | 100.0

**Table 1:** Results of PCR and conventional viral culture.

**Result**  | **PCR**  | **Total**  | **Sensitivity**  | **Specificity**  | **PPV**  | **NPV**
---|---|---|---|---|---|---
Culture  | Positive  | 5  | 5  | 7.46%  | 100%  | 100%  | 57.05%
        | Negative  | 67  | 89  | 156  | 100%  | 100%  |
Total  | 72  | 89  | 161  |  |  |  |

**Table 2:** Sensitivity, specificity, positive predictive value and negative predictive value of virus culture compared to RT-PCR.

The pattern of seasonal variation of picornavirus infections, starting from January 2015 until March 2016 is shown in figure 1. Both HRV and HEV peaked in May 2015, although both were present throughout the study period.

**Citation:** Shereen El Sayed Taha, et al. "Incidence of Picornaviruses among Children with Respiratory Tract Infections Attending Ain Shams Pediatric University Hospital, Egypt". EC Microbiology 14.7 (2018): 414-423.
Table 3 shows that the relation between age and infection with HRVs was not statistically significant, whereas the relation between age and infection with HEVs was statistically significant, with more HEV detections in younger children. Four HRVs were detected in infants aged 1 year old, 15 in children in the age group 2 - 4 years old and 35 were detected in the age group 5 - 10 years old (Table 4). HEVs were detected in 2 children aged 1 year old and in 8 in each of the age groups 2 - 4 and 5 - 10 years old. Overall, a viral cause of ARTI was detected in 100% of infants aged 1 year old, in 51.11% of children aged 2 - 4 years old and in 40% of children aged 5 - 10 years old (Table 4).

<table>
<thead>
<tr>
<th>Virus</th>
<th>Age</th>
<th>Sex</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minimum</td>
<td>Maximum</td>
<td>Mean</td>
</tr>
<tr>
<td>Rhino virus</td>
<td>Negative</td>
<td>1.00</td>
<td>10.00</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>1.00</td>
<td>9.00</td>
</tr>
<tr>
<td>Entero virus</td>
<td>Negative</td>
<td>1.00</td>
<td>10.00</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>1.00</td>
<td>7.00</td>
</tr>
<tr>
<td>Student t test = 2.88 P value = 0.004 (HS)</td>
<td>Chi Square test = 0.85 P value = 0.36 (NS)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Relation between Picornavirus infection and age and gender.
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<table>
<thead>
<tr>
<th>Virus</th>
<th>One year N (6)</th>
<th>2 - 4 year N (45)</th>
<th>5 - 10 year N (110)</th>
<th>All ages N (161)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhinovirus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
<td>30</td>
<td>75</td>
<td>107</td>
</tr>
<tr>
<td>Positive</td>
<td>4</td>
<td>15</td>
<td>35</td>
<td>54</td>
</tr>
<tr>
<td>Enterovirus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>4</td>
<td>37</td>
<td>102</td>
<td>143</td>
</tr>
<tr>
<td>Positive</td>
<td>2</td>
<td>8</td>
<td>8</td>
<td>18</td>
</tr>
</tbody>
</table>

Table 4: Distribution of Picornavirus infection by age group.

There was no statistically significant relation between gender and clinical diagnoses (Chi Square test = 1.23, p value = 0.75). Moreover, we did not find a statistically significant difference between picornavirus infection and gender (Table 3). With respect to the clinical diagnoses, HRVs and HEVs were detected in 39/103 (37.86%) and 12/103 (11.65%) from children diagnosed with acute bronchitis respectively, whereas they were detected in 13/37 (35.14%) and in 6/37 (16.22%) samples from children diagnosed with other URTI. Only one HRV was detected in samples from children diagnosed with pneumonia (1/6; 16.7%) and one in samples from children diagnosed with atypical pneumonia (1/15; 6.7%).

A total of 13 unique samples were used for further virus characterization, by sequencing the 5'UTR and VP4/VP2-regions of the viral genome. Of these, 8 (57.14%) were characterized as HRV-C, 4 (28.58%) were characterized as HRV-A, 1 (7.14%) was characterized as human coxsackie virus A2 and 1 (7.14%) was characterized as human enterovirus C105 (Table 5). HRV-C virus sequences from 4 children were genetically identical (samples 11, 13, 14, 16) and in 6/37 (16.22%) samples from children diagnosed with other URTI. Only one HRV was detected in samples from children diagnosed with pneumonia (1/6; 16.7%) and one in samples from children diagnosed with atypical pneumonia (1/15; 6.7%).

Sample # | Region | Closest match (Genbank ID) | Sequence identity (%) | Best matching virus species |
<table>
<thead>
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</thead>
<tbody>
<tr>
<td>7S</td>
<td>5' UTR</td>
<td>JX129395.1</td>
<td>218/220 (99%)</td>
<td>Rhinovirus C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MF775365.1</td>
<td>584/605 (97%)</td>
<td>Rhinovirus C</td>
</tr>
<tr>
<td>10S</td>
<td>5' UTR</td>
<td>KR997882.1</td>
<td>370/378 (98%)</td>
<td>Rhinovirus C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KR997882.1</td>
<td>583/600 (97%)</td>
<td>Rhinovirus C</td>
</tr>
<tr>
<td>12S</td>
<td>5' UTR</td>
<td>KR675506.1</td>
<td>365/374 (98%)</td>
<td>Rhinovirus C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KR997881.1</td>
<td>562/585 (96%)</td>
<td>Rhinovirus C</td>
</tr>
<tr>
<td>15S</td>
<td>5' UTR</td>
<td>JN798575.1</td>
<td>362/365 (99%)</td>
<td>Rhinovirus A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JN798582.1</td>
<td>581/591 (98%)</td>
<td>Rhinovirus A</td>
</tr>
<tr>
<td>17S</td>
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<td>MF422536.1</td>
<td>281/289 (97%)</td>
<td>Coxsackievirus A2</td>
</tr>
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<td></td>
<td>MF422536.1</td>
<td>544/600 (91%)</td>
<td>Coxsackievirus A2</td>
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<tr>
<td></td>
<td></td>
<td>KR671712.1</td>
<td>448/455 (98%)</td>
<td>Rhinovirus C</td>
</tr>
<tr>
<td>18S</td>
<td>5' UTR</td>
<td>KM880100.1</td>
<td>370/374 (99%)</td>
<td>Human enterovirus C105</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KM880100.1</td>
<td>614/626 (99%)</td>
<td>Human enterovirus C105</td>
</tr>
<tr>
<td>11a</td>
<td>5' UTR</td>
<td>KY624849.1</td>
<td>373/376 (99%)</td>
<td>Rhinovirus C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KY624849.1</td>
<td>585/598 (98%)</td>
<td>Rhinovirus C</td>
</tr>
<tr>
<td>12a</td>
<td>5' UTR</td>
<td>KY698741.1</td>
<td>385/387 (99%)</td>
<td>Rhinovirus A</td>
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<tr>
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<td></td>
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<td>489/497 (98%)</td>
<td>Rhinovirus A</td>
</tr>
<tr>
<td>13a</td>
<td>5' UTR</td>
<td>KY624849.1</td>
<td>370/373 (99%)</td>
<td>Rhinovirus C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KY624849.1</td>
<td>571/583 (98%)</td>
<td>Rhinovirus C</td>
</tr>
<tr>
<td>14a</td>
<td>5' UTR</td>
<td>KY424849.1</td>
<td>369/372 (99%)</td>
<td>Rhinovirus C</td>
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<td>567/579 (98%)</td>
<td>Rhinovirus C</td>
</tr>
<tr>
<td>16a</td>
<td>5' UTR</td>
<td>KY624849.1</td>
<td>367/369 (99%)</td>
<td>Rhinovirus C</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>474/484 (98%)</td>
<td>Rhinovirus C</td>
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<td>17a</td>
<td>5' UTR</td>
<td>KY624849.1</td>
<td>363/364 (99%)</td>
<td>Rhinovirus A</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>560/569 (98%)</td>
<td>Rhinovirus A</td>
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<tr>
<td>18a</td>
<td>5' UTR</td>
<td>KY698741.1</td>
<td>368/369 (99%)</td>
<td>Rhinovirus A</td>
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<td>KY698741.1</td>
<td>582/591 (98%)</td>
<td>Rhinovirus A</td>
</tr>
</tbody>
</table>

Table 5: Species identified by sequencing of the 5'UTR and VP4/VP2-regions of the viral genome.

*Viruses in samples 11, 13, 14 and 16 were genetically identical

*Viruses in samples 12, 17 and 18 were genetically identical

Two viruses were detected in sample 17

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Discussion

Respiratory infections are major causes of morbidity and hospitalizations in children, of which a significant proportion are caused by viruses [16]. HRVs and HEVs are prominent causes of respiratory disease [3]. Rapid identification of viral etiology is critical to avoid unnecessary antibiotics, to initiate antiviral treatment when available and to limit the spread of the infection [17]. The use of molecular diagnostic assays increased the frequency of identification of a respiratory viral infection in children with an acute respiratory condition [18].

In this study, the RT-PCR assay had a higher detection rate than culture of HRV and HEV respiratory infections (33.5% vs. 0.6%; 11.2% vs. 2.5%) respectively. This increase in detection by molecular methods was in accordance with studies performed by Billaud., et al [19], van de Pol., et al [20] and Do., et al [21]. Also, HRVs were detected in 54 out of 161 children (33.5%) with ARTI and HEVs in 18 (11.2%). Numerous studies performed, e.g. by Miller., et al [22], Chung., et al. [23], Leung., et al [24], Stover and Litwin [25], Gökçe., et al [26] and by Fall., et al [27] have observed similar detection rates of picornaviruses as causative agents of ARTI. However, a higher percentage of detection of picornaviruses was reported by Zhang., et al [28], Oue’draogo., et al [29], Visseaux., et al [30] and a lower percentage of detection was found by Kenmoe., et al [31]. Variable outcomes among studies may reflect differences in diagnostic techniques, the clinical criteria applied to the study populations, the panels of detected viruses, and variation in epidemiological patterns. These heterogeneities render comparisons among published studies difficult, if not altogether misleading.

It was demonstrated here that both HRV and HEV infections peaked in May 2015, but were present throughout the study period from January 2015 until March 2016. This result was concordant with results of Miller., et al [22], Fu., et al [32], Fabbiani., et al [2], Stover and Litwin [25] and Bhuyan., et al [33]. In contrast, in studies done by Manoha., et al [34] and Gökçe., et al [26] it was shown that HRV cases peaked in the winter season. These authors attributed the observed patterns to spreading of infectious pathogens and pathogen transmission as a consequence of host behavior due to different meteorological conditions and ultraviolet light radiation that could affect spread by inactivating viruses.

The calculated sensitivity, specificity, positive predictive value and negative predictive value of viral culture considering RT-PCR as the gold standard were 7.46%, 100%, 100% and 57.05% respectively.

Loens., et al [35] found that sensitivity, specificity, positive predictive value, and negative predictive value of culture were 44.7, 100, 100, and 99.8% and those of NASBA and RT-PCR were 85.1, 98.3, 83.3, and 98.5% and 82.9, 93.4, 55.7, and 98.2% respectively, based on the expanded gold standard. Rand., et al [36] compared FilmArray RP (FA) and xTAG RVP (xTAG) versus PCR-confirmed results and found that the sensitivity of FA and xTAG (95.6% and 91.1%), specificity (100% and 100%), negative predictive value (97.8% and 97.5%) and positive predictive value (100% and 100%) respectively. The observed differences could be due to differences in gold standards used, as these studies depend on expanded gold standards that are generally taken to mean both culture and nucleic acid-based methods.

There was no statistically significant relation between gender and both clinical diagnosis and picornaviruses detected in this study. This was in agreement with studies done by van de Pol., et al [20], Fu., et al [32], Zhang., et al [28] and Al-Ayed., et al [37]. Picornaviruses were detected in 9.5% of LRTI and in 50.71% of URTI. This was in accordance with a study performed by Al-Ayed., et al [37] who reported that HRVs (17/22, 77.3%) were associated with URTI’s and 22.7% of HRV were associated with bronchiolitis (4 cases) or pneumonia (one case). Different rates of detection found by Fabbiani., et al [2] who reported that picornaviruses were the most prevalent viruses causing URTI (26.5%) and in (23.9%) led to LRTI. Furthermore, Sentilhes., et al [38] found that HRVs and HEVs were detected in 33% of patients with bronchitis or asthma, in 25% of patients with bronchiolitis, and in 16% of those presenting with pneumonia. These differences could be due to differences in the clinical criteria applied to the study population, the number of patients presenting with URTIs or LRTIs, the panel of detected viruses, and the overall levels of virus circulation locally.

The present study showed that the relation between age and infection with HRVs was not statistically significant, whereas that between age and infection with HEVs was found to be statistically significant. A similar lack of relation between age and infection with HRVs was found in other studies done by van de Pol, et al. [20] and Fall, et al [27].

The majority of picornaviruses detected in this study were in children of the age group 5 - 10 years old. However, Fry, et al. [39] reported that the frequency of HRV detection was highest especially among young children aged < 5 years. Also, Stover and Litwin [25] showed the highest prevalence in the 3-6 age group. This could be explained by differences in the large number of children in certain age groups enrolled in different studies.

It was found in the present study that the most frequently detected species of HRVs and HEVs in the sequenced samples (n = 13) was HRV-C followed by HRV-A, and then HEVs A and C. Similar findings were reported by Linsuwanon, et al. [13] as they found that most of the HRV positive samples were HRV-C followed by HRV-A and HRV-B. This was in contrast to Daleno, et al. [40] who found that the most frequently detected HRV species was HRV-A followed by HRV-C and HRV-B. However, Calvo, et al. [41] found that HRV-B was predominant. This contradiction could be due to differences in the numbers of tested samples, in observation periods, and local differences in epidemiology.

Conclusion
Picornaviruses were responsible for a high proportion of ARTIs among the studied children. RT-PCR was superior in rapidity and improvement of the laboratory diagnoses of ARTI, compared with virus isolation and is thus recommended as the primary diagnostic tool for HRV and HEV infections to increase the diagnostic yield. HRV and HEV were present throughout the year, with a small peak in May.

Bibliography


