Investigation of Respiratory Tract Pathogen Human Parechoviruses in Konya, Turkey

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Abstract

Objective This study aims to better understand the epidemiological characteristics of parechoviruses and to determine the genotype distribution in Konya, Turkey.

Methods In this study, nasal and throat swab samples taken from 1,110 children who were diagnosed with respiratory tract infection (bronchitis, pneumonia, asthmatic bronchitis, and other respiratory diseases) and applied to various pediatric polyclinics of Meram Medical Faculty Hospital, Necmettin Erbakan University between September 2017 and March 2019 were evaluated. Human parechovirus (HPeV) RNA was investigated by multiplex polymerase chain reaction (PCR) in respiratory tract samples. Specific genotypes of the positive samples were identified by real-time PCR amplification of the VP1 region followed by sequence analysis.

Results Of the total of 1,110 samples, 4 were positive for HPeV. Of these, HPeV1 was the most predominant genotype ($n = 3$), followed by HPeV4 ($n = 1$). HPeV infections were detected throughout the year in Konya, Turkey.

Conclusion Although the number of positive samples for HPeV is low, these findings provide information about the genetic diversity and epidemiological of HPeV genotypes circulating in pediatric patients in Turkey. This is the first study to detect prevalence and genotyping of HPeV in respiratory tract infections in Turkey. HPeVs should be considered as causative agents especially in infants with sepsis, meningitis, or encephalitis, and routine testing panels for HPeV detection should be available in hospital laboratories. Further studies using molecular epidemiological methods will be beneficial for identifying genotypes of all HPeVs involved in the etiology and for better monitoring of these infections.

Keywords
- genotyping
- HPeV
- respiratory tract infection
- RT-PCR

Introduction

Human parechovirus (HPeV) is a nonenveloped, single-stranded, positive-sense RNA virus in the *Parechovirus* gene in the family Picornaviridae. When HPeVs were first identified, they were classified as echovirus 22 and echovirus 23 in the *Enterovirus* gene based on cell culture characteristics. Phylogenetic analyzes have shown that these viruses are genetically different from *Picornavirus* genera,[1,2] and subsequently, these viruses were reclassified as *Parechovirus*.[3] HPeV1, HPeV2, and HPeV4 to 8 mainly cause mild gastrointestinal or respiratory diseases, while HPeV3 is associated with severe infections such as sepsis in infants with syndromes and central nervous system infections. HPeV8 to 19 cause gastrointestinal illness, encephalitis, and acute flaccid paralysis in children.[4–7] These genotypes are usually defined as those with <3 months of age, with high hospitalization rates and prolonged hospitalization periods.[8–12] Like other members of the Picornaviridae family, HPeV first replicates in the small intestine and is transmitted through the fecal–oral route. However, replication also occurs in the respiratory system and HPeVs can also be found in respiratory secretions.[13]

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Over the last decade, HPeVs have been shown to be quite common worldwide. Studies in Europe and the United States have generally reported HPeV prevalence of 1 to 7% (i.e., viral RNA or infectious virus prevalence in clinical or surveillance samples), while high prevalence of HPeV in Asia has been reported as 25%.[14] HPeV infections can occur throughout the year. The rate of infections varies between geographic regions and genotypes. There is no report about HPeVs that cause respiratory tract infection in Turkey. Thus, this study aims to understand the epidemiological characteristics of HPeV and to determine the genotype distribution in Turkey.

Methods

Sample Collection

A total of 1,110 respiratory tract samples were collected from patients diagnosed with respiratory tract infection (bronchitis, pneumonia, asthmatic bronchitis, and other respiratory diseases) from pediatrics outpatients of Meram Medical Faculty, Necmettin Erbakan University, Konya between September 2017 and March 2019. All samples were stored at −80°C until they were ready for analysis. The study was approved by Necmettin Erbakan University Ethics Committee.

Viral RNA Extraction, RT-PCR Amplification, and Nucleotide Sequencing

Isolation of RNA from the respiratory tract samples is based on the manufacturer's instructions for EZ1 Virus Mini Kit v2 (Qiagen, Inc., Hilden, Germany).[15] Before detection, viral RNA was reverse transcribed into complementary DNA, using a reverse transcription system (Promega, Madison, Wisconsin, United States). Reverse transcription polymerase chain reaction (RT-PCR) analysis of the HPeV 5′ untranslated region (UTR) and *VP1* gene were conducted simultaneously for HPeV determination and typing using the primer sets: 5′UTR-F1 (5′-CCA CGCTYGTGG AYC TTATG-3′) and 5′UTR-R1 (5′-GGC CTT ACA ACT AGT GTT TGC-3′) and VP1-F1 (5′-CCR RAA YTC RTG GGG YTC-3′) and VP1-R1 (5′-TCYARYTGRTAYACAYKSTCTCC-3′). The PCR products were analyzed by electrophoresis on a 1.5% agarose gel, then extracted for sequencing.[16–18] The HPeV VP1 amplicons were gel purified using the Wizard SV Gel and PCR Clean-Up System (Promega, Fitchburg, Wisconsin, United States). Direct sequencing was then conducted using the ABI PRISM Big Dye Terminator cycle sequencing kit (Applied Biosystems, Waltham, Massachusetts, United States) on an ABI Genetic Analyzer 3130 (Applied Biosystems). We performed genotyping by BLAST (Basic Local Aligment Search Tool) search (National Center for Biotechnology Information [NCBI]) to confirm the analyzed sequences as derived from HPeV.

Phylogenetic Analysis of Molecularly Characterized Isolates

Sequence comparisons were performed using the BLAST in the NCBI GenBank database. As a result of the comparison, species and genus level identification was performed and the sequence data of the identified strains were obtained by accession numbers in NCBI GenBank. Tamura–Nei neighbor-joining method was used for the construction of phylogenetic tree by Molecular Evolutionary Genetics Analysis (MEGA) software version 7.0.[19,20] Confidence levels of the clades were estimated from bootstrap analysis (1,000 replicates).[21]

Results

From September 2017 to March 2019, a total of 1,110 pediatric patient samples were tested for HPeV viral infections at Microbiology Laboratory of Meram Faculty of Medicine. HPeV infection was identified by isolation of the virus from the throat and nasal samples. HPeV indicated that PCR results showed positivity for HPeV 5′ UTR, VP1 region amplified with the VP1 primer set for sequencing. The HPeV genotypes were HPeV1 ($n = 3$) and HPeV4 ($n = 1$).

In our study, all of the HPeV-positive children were between 0 and 4 years of age and we could not find an adult with HPeV infection. The clinical findings of HPeV-positive children are summarized in ►**Table 1**. The monthly distribution of HPeV infections did not show a significant association with HPeV incidence in the season. However, we determined more HPeV infection tendency in autumn months. The VP1 region was fully sequenced in three patients infected with HPeV1 and from one patient infected with HPeV4. A phylogenetic tree was constructed according to the maximum likelihood using MEGA (version 7) with a bootstrap value of 1,000 (►**Fig. 1**).

Table 1 Clinical characteristics of patients with HPeV positivity

Patients		Age (y)	Gender	Clinical symptoms	Diagnosis	Duration of hospital	Medical history
HPeV VP1	Patient 1	4	Male	Throat ache Redness of hands and feet	Allergic rhinitis	–	Diabetes mellitus
	Patient 2	2	Female	High fever Cough	Upper respiratory tract infection	–	Protein energy malnutrition
	Patient 3	1	Male	High fever Cough Respiratory distress	Pneumonia Acute respiratory failure	5 d	Immune deficiency
HPeV VP4	Patient 4	4	Male	Cough Subfebrile fever	Upper respiratory tract infection	–	Endocrine disorder

Abbreviation: HPeV, human parechovirus.

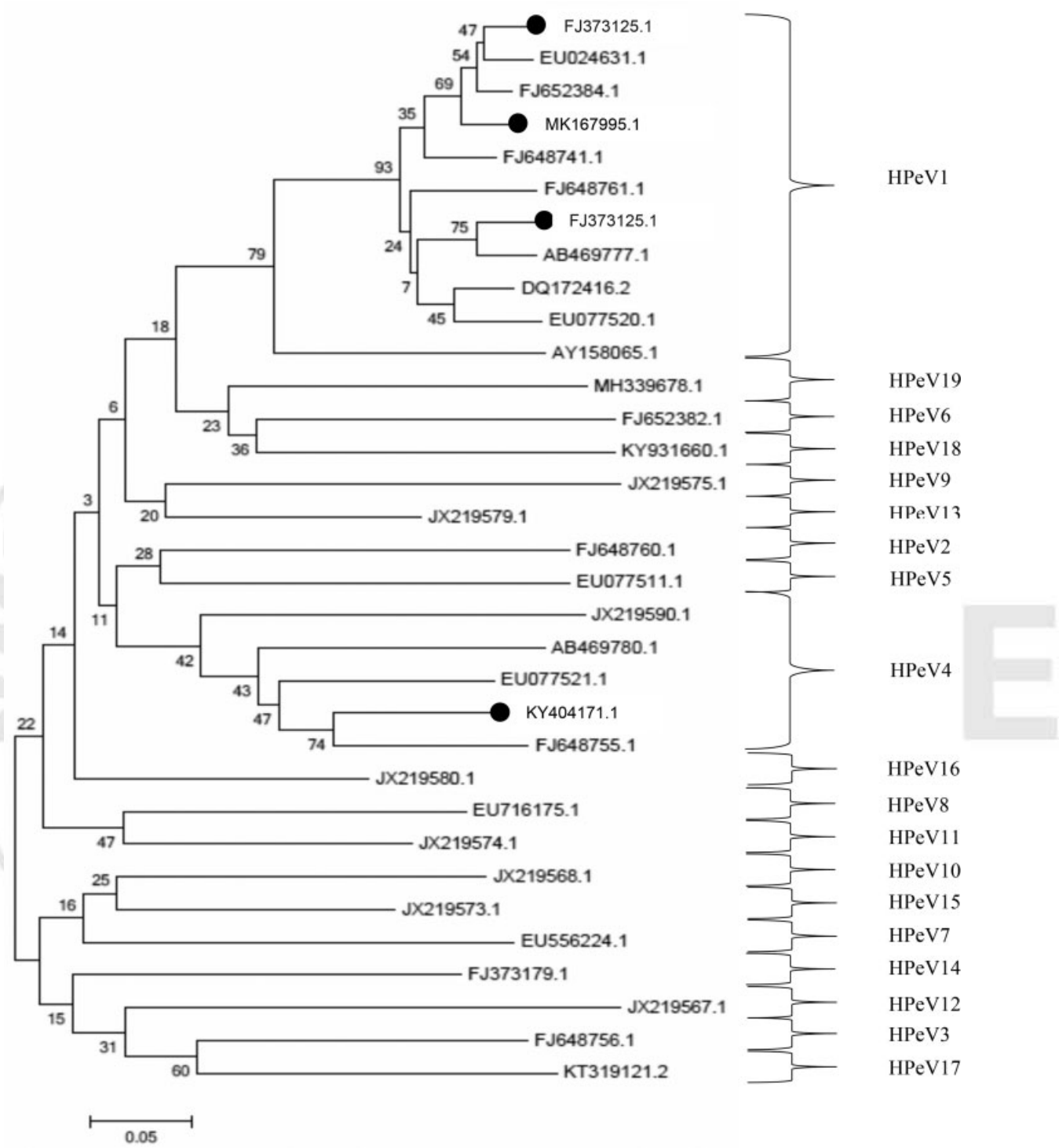

Fig. 1 Phylogenetic trees including our study strains and selected reference strains from the GenBank database. Phylogenetic analysis was performed on the VP1 sequence alignment and on the VP1 sequence alignment using the neighbor-joining (NJ) method (p-distance, 1,000 bootstrap replicates). Isolated viruses in this study are indicated by filled black circles. HPeV, human parechovirus.

Discussion

HPeVs are common pathogens worldwide that infect small children and are transmitted by the fecal–oral route. Most HPeV infections are associated with mild respiratory tract infections and gastroenteritis; however, some HPeV infections are associated with many serious diseases such as sepsis-like disease, myocarditis, encephalitis, meningitis, and stroke. In this study, we found positivity for HPeV in only four children. Except one, other children had signs of mild respiratory infection. One of the children with HPeV had hospitalization due to acute respiratory failure. But this child was also followed for immunodeficiency. Therefore, hospitalization is thought to be related to immunosuppression rather than HPeV isolation. Other children were treated as outpatients. All four HPeV-positive children had a significant

clinical diagnosis in their medical history. None of the children had gastroenteritis.

HPeV1 and 2 are the most well-known genotypes and are associated with mild respiratory tract infection and gastroenteritis.[22,23] It has been shown that PCR is more susceptible than cell culture, especially different clinical samples with low volume and viral load such as cerebrospinal fluid, and some HPeV types are difficult to cultivate.[23–27] In this study, HPeVs were differentiated by RT-PCR amplification method and specific primers from 5′UTR region in viral genome. It has been shown that genotyping of HPeVs is based on VP1 sequence and is related to serotyping.[25–28] In this study, the VP1 binding site was amplified and then sequenced.

The HPeV1 genotype is known as the main HPeV genotype in the world.[22,29,30] Previous studies have shown that HPeV1 is more common in respiratory or gastrointestinal diseases and HPeV3 is more common in sepsis-like diseases and aseptic meningitis.[30–35]

In another study, HPeV3-infected children were significantly younger than those infected with HPeV1; however, this may be related to the presence of maternal antibodies to HPeV1 but not for HPeV3.[29–31,35] In this study, HPeV infection was determined in children younger than 4 years and was consistent with many previous studies.

Although HPeV4 cannot be found in HPeV-related neonatal disease studies, HPeV4 may represent one of the causal agents not detected.[36–38] In addition to fever,[17] HPeV4 has previously been associated with respiratory and gastrointestinal symptoms.[39] In our study, HPeV4 was detected in a patient with mild respiratory symptoms such as subfebrile fever and cough. It is not possible to establish a relationship between the disease and the serotype, since only a small number of positivity was obtained in this study. However, conditions that suppress the immune system, such as diabetes and malnutrition, may pose a risk for parechovirus infection.

HPeV infections are usually seen throughout the year but exhibit an important seasonal epidemiology. It is the highest in the world in autumn and winter.[40] In our study, three of the four genotyped samples were taken in autumn and winter, and they were compatible with the studies.

Conclusion

In our study, the samples identified as positive were sequenced, and three of these samples are HPeV1 and one is HPeV4. These results, we have achieved in Turkey, are important in terms of being the first data on the epidemiology of the HPeV. This virus is an increasingly common cause of morbidity in infants. This increase is probably due to the use of highly sensitive molecular techniques to detect changes in viruses and epidemiology of the disease (changes in virulence or changes in the incidence of the virus). HPeVs should be considered in neonates and especially in young children with sepsis, meningitis, or encephalitis, and cerebrospinal fluid, blood, respiratory, and stool samples should be asked by the clinician in the evaluation of a HPeV suspected febrile infant. Molecular diagnostic methods are necessary for early diagnosis and should be applied as a standard examination for sepsis and meningitis in neonates and infants. As a result, the detection of HPeV in infants will minimize unnecessary extensive medicine and hospital stay, thus reducing the clinical, economic, and public health use of antimicrobials.

Conflict of Interest
None declared.

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