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Identification of human parechovirus genotype, HPeV-12, in a paralytic child with diarrhea

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ABSTRACT

Background: New genotypes of human parechoviruses have been readily identified after improvement of diverse diagnostic tools. We hereby report the detection of a new genotype, HPeV 12, from a child presented with diarrhea and paralysis.
Objectives: The genetic variability of human parechoviruses has recently expanded defining 16 genotypes however data available covers only 11 genotypes. The present study was designed to determine the genetic characterization of human parechovirus identified in a child with gastroenteritis and acute flaccid paralysis (AFP).
Study design: Stool samples are referred to Virology Department, NIH-Pakistan for the routine detection of enteroviruses and polioviruses through cell culture and RT-PCR. Five of isolates showing cytopathic effect on L20B cell line but negative for poliovirus were further explored for human parechovirus using multiple cell lines and RT-PCR.
Results: Human Coxsackie A virus type 2, 3, 6 and 20 were found in four samples whereas the fifth sample contained human parechovirus genotype 12. Efficient growth of human parechovirus was found on L20B cells while Vero and LLC-MK2 cells showed no apparent cytopathic effect.
Conclusions: This study describes the detection of a new human parechovirus genotype (HPeV-12) in a paralytic child with diarrhea. Human parechoviruses are now considered as potential pathogens that may cause a number of serious clinical complications especially in infants and young children. These findings emphasize to conduct large scale epidemiological surveys in the country to understand their association with clinical diseases especially gastroenteritis, respiratory and neurological disorders.

1. Background

Human parechovirus (HPeV) belongs to family picornaviridae with single stranded positive sense RNA genome.[1,2] The family comprises of 12 genera including the three; hepatovirus, enterovirus and parechovirus, mostly associated with human infections.[3] These viruses, originally known as echovirus 22 and 23, were reclassified in 1999 as a separate genus, parechovirus, based on the differences in their structural as well as biological properties.[2,4] The RNA genome of parechoviruses is comprised of a single open reading frame (ORF) with 5′ and 3′ untranslated regions. The single polyprotein translated by ORF is finally expressed as three structural capsid proteins (VP0, VP3, VP1) and seven nonstructural proteins (2A, 2B, 2C, 3A, 3B, 3C and 3D) with majority of antigenic determinants identified in the N-terminal region of VP0 protein.[5,6]

HPeVs are known to have worldwide prevalence and have been isolated from patients with multiple clinical presentations including gastroenteritis, hand-foot-mouth disease, upper respiratory tract infections, bronchitis, rashes, aseptic meningitis, Reyes's syndrome, neonatal sepsis-like illness, myocarditis, encephalitis, otitis media, myositis, fever of unknown origin and flaccid paralysis.[3,7,8] HPeV-1 was associated with an AFP outbreak in Jamaica in 1986. HPeV-3 was isolated from a child with transient paralysis.[9] HPeV-1, -5, -6 and -7 have been detected in patients with non-polio AFP.[10] Human parechovirus-4 has been detected in a child with high fever.[11] HPeV-2 and HPeV-6 have been suggested to cause gastroenteritis in children[12] although HPeV-6 has also been detected from the stool specimen of an AFP patient in Japan during 2001.[7] HPeV-7 was found in stool sample from a healthy boy having close contact with a person who had non-polio acute flaccid paralysis in Pakistan.[10] HPeV-8 was isolated from a child with enteritis in

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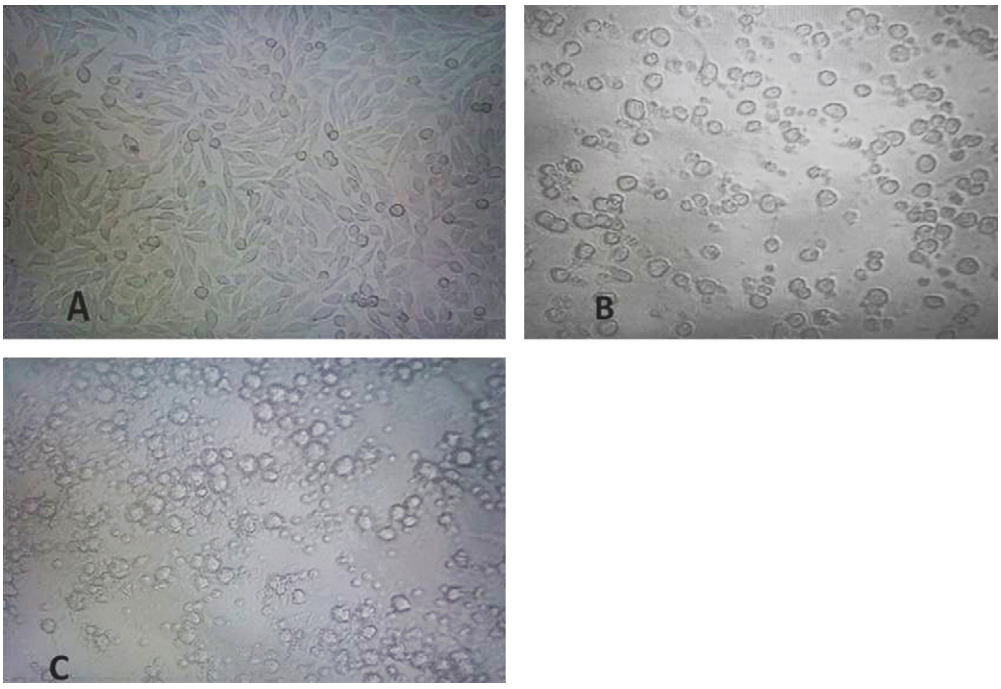

Fig. 1. Cytopathic effect of PAKNS911A-2011 on L20B cell line. (A) Normal L20B cell confluent monolayer maintained under minimum essential medium with Hank's balanced salt solution and 2% fetal bovine serum (B) shows early CPE after 24 hours with rounded, enlarged cells. (C) The cytopathic effects after 120 hours (5 days) of incubation with cells still adherent to the tube surface although cell lysis and degeneration signs are apparent.

Brazil.[13] Recently, HPeV-11 has also been detected in Sri Lanka from patients with acute gastroenteritis.[14] Although HPeV infections are common among children, the data related to infection in adults is very scant.[15]

2. Objectives

Many recent reports have identified new parechoviruses and thus the total number of genotypes identified so far is 16 (HPeV 1–16) although there is published data available for only 11 genotypes, i.e. type 1–8, 10, 11, and 14.[14,16] We hereby describe the identification of a new, previously unpublished, genotype designated as HPeV-12, found in a child with acute flaccid paralysis and diarrhea.

3. Study design

Samples collected from patients affected by acute flaccid paralysis are referred to Department of Virology, National Institute of Health, Islamabad, Pakistan for the detection of polio- and enteroviruses as a part of Polio Eradication Initiative program. These stool samples are processed and inoculated on two continuous cell lines (L20B and RD) for the isolation of enteroviruses. Five of the randomly selected samples that showed cytopathic effect (CPE) on L20B, but found negative for polioviruses and having watery diarrhea like consistency, were inoculated on additional cell lines like African Green Monkey Kidney (Vero) and tertiary monkey kidney (LLC-MK2) to rule out the presence of human parechovirus.

RNA extracted from L20B culture fluid using QIAamp viral RNA minikit (Qiagen, GmbH, Germany) was tested for the detection of human parechoviruses using RT-PCR targeting 5′UTR as described previously.[17] Thereafter, to determine the HPeV genotype, viral protein 1 gene was amplified using a nested PCR as described.[18] and the amplified product of round 2 was directly sequenced in both directions with the primers VP1-parEchoF1 and VP1-parEchoR1 using the BigDye Terminator Cycle Sequencing kit v3.1 (Perkin Elmer-Applied Biosystems, Inc.). The sequence reads of VP1 gene obtained through ABI Prism 3100 Genetic Analyzer (Perkin Elmer-Applied Biosystems, Inc.) were edited using Sequencher v.4.1 (Gene Codes Inc., Ann Arbor, MI, USA). The phylogenetic tree was reconstructed using MEGA 4.0.

Alternatively, to achieve the HPeV growth through cell culture, the samples were again re-grown on L20B cells to increase the infective viral titer and then passaged to LLC-MK2 and Vero cells, known to be susceptible for HPeV[19] and were observed for initial 9 days until cells degeneration started. 200 μl of cell culture lysate was re-passaged on the two respective cell lines but no apparent CPE was observed even after 10 days (19 days of total cell culture).

4. Results

The samples showing non-enterovirus like CPE, i.e. enlarged, swollen cells that remained attached to the culture tube surface on L20B cells (Fig. 1) were further inoculated on two additional cell lines, Vero and LLC-MK2, that are known as the suitable in vitro host for parechoviruses but none of the cell line yielded any apparent cytopathic effect uptil 19 days of total observation.

Four samples were found positive for human coxsackievirus A viruses (CAV-2, -3, -6 and -20), whereas, one of the sample was found positive for human parechovirus through RT-PCR. The online BLAST searches of VP1 sequence through GenBank database showed the closest identity of PAKNS911A-2011 with human parechovirus type 10 (80% nucleotide and 80.5% aminoacid identity). The sequence data was submitted to the picornavirus study group (http://www.picornastudygroup.com/) for type identification. The study virus PAKNS911A-2011 was designated as HPeV-12, as a new unpublished genotype of HPeV. The nucleotide sequence has been submitted to the GenBank and has been assigned an accession no. JQ513376. The nucleotide and aminoacid identities were found as 86% and 98.5% respectively with the HPeV-12 prototype strain BAN2004-10904. The phylogenetic tree reconstructed against representative members of all available HPeV genotypes in