Short Communication

Analysis of Human Parechovirus Genotypes in Yokohama District from 2000 to 2016

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SUMMARY: Human parechovirus (HPeV) infections in Yokohama City, Japan, were surveyed from 2000 to 2016. The sequence of the VP1 region of HPeVs was used to construct a phylogenetic tree and to reveal the putative amino acid (aa) sequences. Phylogenetic analysis showed the presence of 3 genotypes in Yokohama City: HPeV1 (25 specimens), HPeV3 (86 specimens), and HPeV4 (2 specimens). HPeV1 was detected nearly every year, with the highest number detected in 2014. HPeV3 was not detected until 2005, but was detected over a 1- or 3-yr period thereafter. HPeV1 was most prevalent from July to November, whereas HPeV3 peaked in July and August each year. HPeV1 was mainly detected in patients with infectious gastroenteritis or respiratory tract infections. In contrast, 87% of HPeV3-positive cases were in patients less than 2 months of age with a viral-induced fever. An analysis of the aa sequence of VP1 revealed a divergence within the same HPeV genotype, which was useful in analyzing the emergence and re-emergence of HPeV infections during the survey period. These findings suggest that molecular analysis of HPeVs may contribute to a better understanding of its epidemiology.

The human parechovirus (HPEV), a member of the Picornaviridae family, has a single-stranded ribonucleic acid (RNA) genome of approximately 7.3 kb in length that contains an open-reading frame from which all virus proteins are produced (1). HPEVs are currently classified into 17 different genotypes (2). HPEV infections are associated with a wide variety of diseases (3). This study surveyed the incidence of HPEV infection from 2000 to 2016 in Yokohama City, Japan. The frequency and seasonality of viral outbreaks and the genetic variability of HPEVs were determined among the patient specimens. The study was conducted as part of the National Epidemiological Surveillance of Infectious Diseases, Japan, as stipulated under the Infectious Diseases Control Law. The ethics committee of the Yokohama City Institute of Public Health approved this study.

All specimens were supplied by 21 medical institutions in Yokohama district. Specimens of throat swabs or stools were collected from patients exhibiting symptoms of infectious gastroenteritis, respiratory tract infections, and herpangina who were suspected to be HPEV- or enterovirus-infected. The other specimens, including throat swabs, stools, cerebrospinal fluid, or serum, were obtained from hospitalized patients who were suspected to have virus-infected diseases on the basis of the discretion of the attending physician. The total number of patients was 806 and total number of specimens was 976. Specimens or lysates of specimens were inoculated in indicator and Vero cells to recover HPEVs. Viruses were recovered from 26 specimens and were subjected to analysis of their genomes (4–6). The other specimens from which viruses could not be recovered were subjected to extraction of total RNA for the analysis of viral RNA. The presence of viral RNA was analyzed using a reverse transcription polymerase chain reaction (RT-PCR) assay with a primer set that detected the conserved 5’ terminal region of the genome (4). For HPEV-positive specimens, the VP1 region of the HPEV was analyzed using an RT-PCR assay (4–6). Overall, 113 specimens obtained from 79 patients were positive for HPEV infection. Twenty-five samples (8 throat swabs and 17 stool samples) from 25 patients were positive for HPEV1; 86 samples (19 throat swabs, 27 stool samples, 25 cerebrospinal fluid samples, and 15 serum samples) from 52 patients were positive for HPEV3; and 2 stool samples from 2 patients were positive for HPEV4. The sequences were deposited in GenBank with the accession numbers LC133375 to LC133462 and LC259180 to LC259204.

Table 1 shows the yearly and monthly rate of detection of HPEV infection together with clinical characteristics of the patients. HPEV1 infection was sporadically detected throughout nearly the entire period, but its incidence increased after 2010 and peaked in 2014. In contrast, HPEV3 was first detected in 2006 and was detected sporadically thereafter, with 2 peaks in 2011 and 2014. HPEV4 was detected only in 2015. An analysis of the monthly appearance of these viruses revealed that HPEV1 was detected during all months except January and February, peaking from July to October. HPEV3 peaked sharply in July and August, and HPEV4 was detected in May and July. Most patients infected with HPEV1 were diagnosed with infectious gastroenteritis or respiratory tract infections. HPEV3 was detected in 20 patients less than 2 months of age who had been diagnosed with a viral infection-induced fever. HPEV4
was detected in 2 patients with infectious gastroenteritis or a viral infection-induced fever.

The VP1 region was fully sequenced in 23 specimens from 23 patients infected with HPeV1, 74 specimens from 51 patients infected with HPeV3, and 2 specimens from 2 patients infected with HPeV4. There were no patients found with the same VP1 sequence. The VP1 sequence was identical only in multiple specimens from the same patient. A phylogenetic tree was constructed according to the maximum-likelihood using MEGA (version 5) with a bootstrap value of 1000 (Fig. 1). The homology of VP1 nucleotides was 65% (448/693) for HPeV1, 85% (579/678) for HPeV3, and 89% (620/696) for HPeV4. Most of the HPeV1 infections except HPeV1/Yokohama.JPN/47.04/ts in Yokohama City were classified as contemporary strains belonging to designated clade 1B, like many isolates in Japan (6–8). Of note, certain isolates repeatedly emerged within the limited group of HPeV1 clade 1B. The sequences of 58 HPeV3 cases, except for those obtained in 2016, resembled those previously reported in Japan (8–10) that are in the designated clusters as shown in Fig. 1. However, some cases obtained in 2011 (HPeV3/Yokohama. JPN/31.11 and HPeV3/Yokohama. JPN/36.11 etc.) showed a close similarity to those obtained in 2006, and 2 cases in 2014 (HPeV3/Yokohama. JPN/29-1.14 and HPeV3/Yokohama. JPN/33-1.14) showed a close similarity to those obtained in 2011. Seventeen of the 16 HPeV3 cases obtained in 2016 were classified in a new cluster. The 2 HPeV4 cases occurred only 2 months apart. The difference in the sequences of the isolates suggests that these 2 patients were infected from 2 different viral sources.

Next, the putative amino acid (aa) sequences of the entire VP1 sequence in 22 and 74 cases of HPeV1 and HPeV3 were compared, respectively, according to the annual emergence (Table 2). By categorizing aa residues with the same acid-basicity and polarity together, the hot spots of aa variation in the VP1 gene were highlighted. Aa sequence variation was clustered in the center (85th, 137th, and 138th aa) from the N-terminal of VP1 and the C-terminal regions (218th, 226th, and 230th aa) of VP1 in the HPeV1 cases and in the C-terminal region (222nd, 226th, and 229th aa) in the HPeV3 cases. The 85th, 138th, and 230th aa residues were either Asp (D) or Asn (N) in all the HPeVs. The 218th and 226th aa residues were Gly (G)/Ser (S) and Ala (A)/Thr (T), respectively, which were both neutral aa residues with different polarities. The 222nd aa residue in HPeV3 was Arg (R) in all the HPeV1, but was R, G, or Glu (E) (which have different acid-base properties) in HPeV3. Interestingly, the 137th aa in HPeV1 was A, T, or E (which have different aa characteristics), but was D in all the HPeV3 cases. Of note, the aa sequences in the central region (i.e., the 85th, 137th, and 138th aa residues) varied in HPeV1, but were conserved in HPeV3. Furthermore, an aa sequence with an R-G-D motif, which is thought to be an important tri-peptide for virus attachment to cell surfaces, was present at the 222nd–224th position in the VP1 region in all the HPeV1, but this sequence motif did not exist in...
Fig. 1. Phylogenetic relationships among HPeV isolates obtained in this study and previously published sequences based on alignment of the entire VP1 region. The bar indicates the nucleotide substitutions per site. The homologous sequences of HPeV3 VP1 clustered according to the year(s) of detection are shown to the right of (B). ◆: HPeV strains obtained in this study.
the HPeV3 VP1 region (11). The differences in these characteristic aa sequences in the VP1 region between HPeV1 and HPeV3 may contribute to the virological differences underlying these HPeV genotypes.

The analysis of HPeV infection in Yokohama district over a 17-year period showed the presence of HPeV1, -3, and -4, with HPeV3 having the highest prevalence (66%, 52/79), especially after 2006. Nucleotide and putative aa sequence analyses revealed the presence of non-synonymous mutations in the VP1 region of HPeV and contributed to the evaluation of the annual emergence of original or mutated HPeV variants during the analysis period. In particular, some HPeV1 variants detected in 2001 and 2005 and some HPeV3 variants detected in 2006 and 2007 continued to be detected for nearly another 10 years. Importantly, 87% (45/52) of the HPeV3-infected patients were less than 2 months of age, with most being hospitalized. HPeV3 infection is correlated with the onset of severe diseases in newborns and during early infancy, as was confirmed in this study. In addition, as reported by another group (12,13), a few newborns and infants with HPeV1 infection (2 patients with encephalopathy who were less than 6 months of age) and HPeV4 infection (1 patient who was less than 2 months of age) were also observed in this study. These findings suggest that the molecular analysis of HPeV may contribute to a better understanding of its epidemiology.

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Conflict of interest None to declare.

REFERENCES