Human parechovirus type 3 (HPeV-3), a member of the \textit{Parechovirus} genus in the \textit{Picornaviridae} family, was first isolated from the stool specimen of a 1-year-old patient with transient paralysis in 1999 (1). The virus has since been isolated from patients with various diseases such as gastroenteritis, respiratory tract diseases, and exanthematous disease in several countries (2). In the summer of 2008, an HPeV-3 epidemic was observed in Hiroshima City, Japan. Most of the HPeV-3 patients were infants under the age of 4 months. In this report, we describe the virological and epidemiological characteristics of these HPeV-3 infections.

We examined 1,293 clinical samples collected from 876 patients at sentinel clinics or hospitals in Hiroshima City from January to December in 2008. Virus isolation was done with Vero, HEP-2, RD-18S, and human embryonic fibroblast cells. Isolated viruses that caused an HPeV-like cytopathic effect in Vero cells were initially examined by neutralization tests with anti-HPeV-1 antiserum (Denka Seiken, Tokyo, Japan). When the viruses were not neutralized, they were genetically examined. Viral RNAs were extracted from culture suspensions and then reverse-transcribed to cDNAs. A part of the 5´-noncoding region of the cDNAs was amplified by a real-time PCR method (3) that was originally designed to commonly amplify HPeV-1 and HPeV-2, with some modifications. When the result of real-time PCR was positive, a part (812 bp) of the VP1 region that was amplified by RT-PCR (4) was directly sequenced, and 305-bp nucleotide sequences were determined and compared. Phylogenetic analysis was performed by the neighbor-joining method using Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0 with the reference strains of HPeV-1 (accession no., L02971), HPeV-2 (AJ005695), HPeV-3 (AB084913), HPeV-4 (AM235750, DQ315670), HPeV-5 (AF055846, AM235749), and HPeV-6 (AB252582).

HPeV-3 was isolated from 40 (5%) out of 876 patients, accounting for 18% of 225 virus-positive patients in the period studied. Most of the HPeV-3 strains were isolated from throat swab samples (29 strains) or stool samples (24 strains), and 5 strains were isolated from liquor samples (Table 1). In total, HPeV-3 was isolated from 58 (4.5%) out of 1,293 clinical samples. HPeV-3 was only isolated in Vero cells. The HPeV-3 isolates were genetically close to each other, showing homologies of 99.3 to 99.7% in the VP1 region compared, and 95.0 to 96.0% homologies to the HPeV-3 reference strain (Fig. 1). All HPeV-3 isolates were detected only in the period between June and October, and 35 isolates (85%) were detected in the summer season of June to August (Fig. 2). On the other hand, HPeV-1 was sporadically isolated from 5 patients.

All the HPeV-3 patients but 1, whose age was unknown, were infants aged under 2 years and most of them (32 patients) were infants aged under 4 months (Fig. 3), suggesting that the main reason for their infection with HPeV-3 was that they did not receive maternal IgG antibodies from their mothers. Twenty-one HPeV-3 patients were diagnosed as having acute febrile illness (unidentified fever). The clinical diagnoses of the other patients were categorized as respiratory tract diseases (7 patients), aseptic meningitis (5 patients), exanthematous diseases (3 patients), pharyngo-conjunctival fever (2 patients), infectious gastroenteritis (1 patient), and herpangina (1 patient), demonstrating that HPeV-3 is associated with various kinds of diseases, as previously described (2). The main clinical symptom was fever, which was seen in 39 (98%) patients, and 15 patients (38%) had only fever without other apparent symptoms. The other symptoms were gastroenteritis in 7 patients (18%), upper respiratory syndrome in 7 patients (18%), eruption in 5 patients (13%), central nervous system syndrome in 5 patients (13%), and lower respiratory syndrome in 2 patients (5%). One patient had gait disorder and another patient had myocarditis and cardiovascular disorder.

In Hiroshima City, HPeV-3 was first isolated from a patient in 2004. The virus was subsequently isolated from 2 patients in 2005, 26 patients in 2006, 1 patient in 2007, and 40 patients in 2008, while HPeV-1 has been sporadically isolated in from 4 - 6 patients each year over the same period. This difference in the annual detection rates between HPeV-3 and HPeV-1 was also observed in the nationwide surveillance of Japan. In contrast with HPeV-1, which may cause sporadic infections or small epidemics in most years, HPeV-3 may cause large

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline
Sample name & No. of positive samples & No. of samples tested \\
\hline
Throat swab & 29 & 744 \\
Feces & 24 & 325 \\
Liquor & 5 & 126 \\
Urine & 0 & 86 \\
Conjunctival swab & 0 & 4 \\
Cutaneous lesion swab & 0 & 3 \\
Others & 0 & 5 \\
\hline
Total & 58 & 1,293 \\
\hline
\end{tabular}
\caption{Number of HPeV-3 positive samples and samples tested}
\end{table}
epidemics every few years.

In summary, HPeV-3 was isolated from 40 patients in the summer of 2008. Thirty-two patients (80%) were infants aged under 4 months. The main clinical symptom was fever and 15 patients (38%) had only fever without other apparent symptoms.

REFERENCES