Seroepidemiology of human parechovirus types 1, 3, and 6 in Yamagata, Japan, in 2014

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ABSTRACT

To clarify the seroepidemiology of human parechovirus type 1 (HPeV1), 3 and 6, neutralizing antibodies (NT Abs) were measured in 214 serum specimens collected in 2014 in Yamagata, Japan. The seroprevalence against HPeV1 was 100% in all age groups, while that against HPeV3 and HPeV6 was 79.4% and 66.8%, respectively, overall. The geometric mean titers of NT Abs against HPeV1, 3 and 6 were 755.2, 255.0 and 55.9, respectively, overall. Our findings indicate that HPeV1 is the most prevalent HPeV circulating in Yamagata, followed by HPeV3 and HPeV6.

Key words emerging disease, human parechovirus, seroepidemiology.

Human parechoviruses are members of a relatively newly created genus designated Parechovirus in the family Picornaviridae (1, 2). At least 16 types have been proposed to date (1). The two initial types, HPeV type 1 (HPeV1) and HPeV2, were first classified as echovirus types 22 and 23 in the genus Enterovirus, but were later reclassified on the basis of genetic analysis and biological features (1, 2). HPeV3 was first isolated from a stool specimen from a 1-year-old Japanese child with transient paralysis in 1999 (3); it has since been identified worldwide. HPeV6 was first isolated from a cerebrospinal fluid specimen from a 1-year-old girl with Reye syndrome (4). Virological and serological studies have suggested that HPeV infections occur early in life (1, 2, 5, 6), HPeV1 being the most frequently identified member of the genus, followed by HPeV3 (1, 2).

HPeV infections are mainly asymptomatic or associated with mild respiratory and gastrointestinal symptoms; however, more serious diseases, such as meningitis, encephalitis, pneumonia, flaccid paralysis and sepsis-like syndrome, have been occasionally reported in children (1, 5-8). In particular, HPeV3 often causes severe illnesses involving the central nervous system, such as meningitis, encephalitis and sepsis-like syndrome, in young children; cases tend to peak during summer every 2–3 years in Japan and Europe (5–7, 9–12). According to our previous studies, HPeV3 also causes epidemic myalgia and myositis in both adults and children during these summer outbreaks. This phenomenon was also observed in Osaka, Japan, during an outbreak in 2014 and was recently described in a textbook for the first time (1, 13–16). The detection of HPeV6 has been quite limited in comparison with that of HPeV1 and HPeV3, with HPeV6 viruses detected from infants and children with Reye syndrome, gastroenteritis, rash, flaccid paralysis, and upper respiratory infection, according to the original report (1, 4).

In our role as a public health laboratory, both seroepidemiological studies and analysis of viral pathogens are important in improving strategies against viral infectious diseases. Thus, we participated in the NESVPD led by the Ministry of Health, Labor and...
Welfare, Japan by measuring NT Abs against vaccine-preventable diseases such as polio as well as carrying out longitudinal epidemiological studies as part of the NESID, Japan. We also combined the serum specimens collected for the NESVPD and viral isolates collected during our work with the NESID, and performed serological analyses of viruses other than those targeted by the NESVPD (17, 18). We consider this type of combination and testing of serum specimens and viral isolates to be an efficient and necessary approach to clarifying the epidemiology of viral infectious diseases and improving the strategies against them.

Because HPeV3 and HPeV6 infections are both emerging diseases, there have been only a few seroepidemiological surveys concerning them to date. We isolated HPeV 1, 3 and 6 and undertook a seroepidemiological study using our representative isolates and sera collected from residents of Yamagata in 2014. Herein, we discuss our results, together with previously published data.

We collected serum samples from residents of Yamagata from whom we had obtained informed consent (either from the individual or a guardian) between June and October 2014 as part of our work with the NESVPD. There were 214 serum samples (0–4 years, 33; 5–9 years, 31; 10–14 years, 49; 15–19 years, 7; 20–29 years, 7; 30–39 years, 25; 40–49 years, 30; 50–59 years, 22; over 60 years, 10; 94 male and 120 female subjects) in this study. Although we intended to collect specimens from healthy individuals, it was difficult to collect such specimens from children. We therefore collected the specimens in the ≤15 year age group from patients, most of whom had presented to the Yamanobe pediatric clinic with acute respiratory infections. This study was approved by the Ethics Committee of the Yamagata Prefectural Institute of Public Health (YPIPHEC H26-01).

The serological study consisted of measurement of NT Ab titers against HPeV 1, 3 and 6 using a micro-neutralization test with the LLC-MK2-N cell line. When we used this cell line in a previous study, we found that it is sensitive to HPeV3, whereas the LLC-MK2 cell line, which is commonly used, is not (13). Serum samples were inactivated at 56°C for 30 min and then serially diluted two-fold from 1:8 to 1:1024-fold. We mixed and incubated (37°C, 60 min) 50 μL of each diluted serum sample with 60 μL of viral fluid containing 100 times the TCID50 of the representative strains. These strains were 1229-Yamagata-2004 for HPeV1, 1356-Yamagata-2008 for HPeV3, which we had used to measure the NT Ab titers against HPeV3 among adult myalgia patients in 2008 in a previous study (13) and 1343-Yamagata-2004 for HPeV6. We registered sequence data for these challenge viruses under the GenBank accession numbers, LC171401, AB668030 and LC129277, respectively. We prepared confluent monolayers of the LLC-MK2-N cell line in advance using 96-well tissue culture plates, washed the cells with PBS without calcium or magnesium, and inoculated 50 μL of each incubated virus–serum mixture into each of two cultures. The plates were then incubated in a 5% CO2 incubator at 33°C for 72 hr. The CPEs of both cultures at each serum dilution were compared with the CPE of the control cultures and the serotiters determined. The reciprocal value of the highest dilution of each serum resulting in no CPE as compared to the control was taken to be the titer. Seropositivity was defined as a serotiter ≥1:8. Statistical analysis was performed using Pearson’s χ2 test. P values <0.005 were considered significant.

The NT titers and seroprevalence against the representative HPeV strains from Yamagata are shown in Figure 1. The seroprevalence against HPeV1 was 100% for all age groups. To rule out the possibility of false positive reactions, we confirmed that serum specimens having no NT Abs against another HPeV strain (3367-Yamagata-2002) in GMK cell lines in our previous study (19) showed NT Abs of <1:8 in this study. Although seroprevalence against HPeV3 was 51.5% in individuals aged less than 5 years, it was over 70% in the older age groups and the overall positive rate was 79.4%. Seroprevalence against HPeV6 was over 70% in the 10–14 and 20–49 age groups, but between 40% and 70% in the other age groups, giving an overall positive rate of 66.8%. The seroprevalence of HPeV1 was significantly higher than that of HPeV3 and HPeV6 (100% vs. 79.4% and 66.8%, respectively; P <0.0001), whereas that of HPeV3 was significantly higher than that of HPeV6 (79.4% vs. 66.8%; P <0.005). The overall GMTs of NT Abs against HPeV1, 3 and 6 in the study subjects from Yamagata were 755.2, 255.0 and 55.9, respectively. The GMT against HPeV1 was strongly conserved in all age groups, whereas those against HPeV3 and 6 were high in the younger age groups and decreased with age.

We had previously accidentally detected HPeV3 in nasopharyngeal specimens from four children enrolled in this study (14), having collected the serum specimens prior to HPeV3 detection in two children with NT Ab titers <1:8. A 7-year-old child identified as HPeV3-positive on 22 July had a NT Ab titer of 1:2048 on 10 September, and another 2-year-old child, identified as HPeV3-positive on 28 July had a NT Ab titer of 1:1024 on 25 August.

With regard to HPeV1, both seroepidemiological studies performed between 1965 and 2002 and this study, indicated that HPeV1 affects young people and that HPeV1 has been consistently endemic in Japan over...
the last 50 years (19–22). Data from NESID, which show that HPeV1 has been detected every year since surveillance started in 1981, also support the notion that HPeV1 has been endemic over the past few decades in Japan (23). Seroprevalence against HPeV1 is reportedly 99% and 82% in Finland and in the Netherlands, respectively (24), and 97% among adults in Finland in another study (25). Furthermore, HPeV1 was detected every year between 2000 and 2007 in the Netherlands and almost every year between 1965 and 1990 in Sweden (6, 26). These data suggest that HPeV1 has been endemic worldwide for a long period.

In the present study performed on residents of Yamagata, the NT Ab-positive rate against HPeV3 increased with age up to 100% in the 15–19 year age group and then fluctuated between 70%–96% in older age groups (Fig. 1), whereas Ito et al. have reported that the NT Ab positive rate increased with age up to 85% in the 4–6 year age group and then fluctuated between 56.5%–91.3% in older individuals in Aichi, Japan (3). Thus, both sets of data suggest that HPeV3 infections commonly occur in children in Japan. However, the seroprevalence in children is only 10% and 13% in the Netherlands and Finland, respectively; the reason for this discrepancy is unknown (24). In contrast to HPeV1, HPeV3 causes epidemics every 2–3 years, mainly during summer, in temperate climates, including Japan and Europe (6, 12–14, 23, 27). Although we are not sure that the endemicity of HPeV1 and epidemicity of HPeV3 influence their seroprevalence, the results of our seroepidemiological study suggest that HPeV3 infection is likely to be less common than HPeV1 infection.

Given that viral genomes have been detected in serum specimens in the acute phase of HPeV3 infections in both children and adults, viremia may be an important initial step in disease progression (10, 11, 13, 14). Aizawa et al. reported NT Ab titers against HPeV3 of ≤1:16 in 45 neonates and young infants with severe diseases related to HPeV3, and chose a titer of ≥1:32 as the NT titer necessary for preventing infection (28). We have previously reported that NT Ab titers were ≤1:16 in the acute phase in all of 12 adult patients with epidemic myalgia associated with HPeV3 infections from whom serum specimens had been collected within 7 days of the

Fig. 1. Antibody titers and positive rates against HPeV 1, 3 and 6 according to age group in Yamagata, Japan in 2014. The numbers in parentheses under the age groups indicate the GMTs.
onset of illness (13). These data also suggest that a NT Ab titer of ≥1:32 potential protects against viremia in cases of HPeV3 infection. When we took a titer ≥1:32 to indicate seropositivity and compared that with a titer ≥1:8, the NT Ab-positive rates declined slightly by 0–8% in individuals aged less than 40 years, whereas they declined markedly from 70%–86.4% to 20%–63.3% in older individuals (Fig. 1). These findings are similar to those for seroprevalence for HPeV3 in cord blood samples, which has been shown to decline with increasing maternal age (28). Westerhuis et al. reported high concentrations of NT Abs against HPeV1 1 year after infection, whereas they detected no NT Abs against HPeV3 after the same interval (29). These findings raise the question of whether HPeV3 infection confers lifelong protection, as previously suggested (28). We have reported that most adult patients with myalgia are aged between 20 and 40 years; that is, are of child-rearing age (13–15) and our seroepidemiological analysis clearly indicates that some individuals of child-rearing age have no or low NT Ab titers (≤1:16). From these data, we speculate that children of mothers without sufficient protecting NT Abs are likely to be susceptible to HPeV3 infection; others have already suggested this is so (28).

In Finland and the Netherlands, the seroprevalence against HPeV6 was reported as 74% and 57% (24), respectively; these values are similar to those found for Yamagata in this study. However, only limited data related to HPeV6 is currently available. For example, no national data are currently available from the NESID in Japan and HPeV6 is detected considerably less frequently than HPeV1 and 3 (5, 9). Thus, further studies are necessary to define the epidemiology of HPeV6.

Our study indicates that HPeV1 is the most prevalent HPeV type in Yamagata, followed by HPeV3 and HPeV6. Based on a results of detection survey using a PCR method rather than a seroepidemiological study, Tapia et al. have also reported that HPeV1 is the most prevalent type (76%), followed by HPeV3 (13%) and HPeV6 (9%) (30). Thus, most available data support the endemicity of HPeV1 as described above, whereas further studies are necessary to clarify the epidemiology of HPeVs such as HPeV3 and 6, which have recently appeared as emerging viruses.

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DISCLOSURE

The authors declare no conflict of interest.

REFERENCES


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