Laboratory and Epidemiology Communications

An Outbreak of Parainfluenza Virus Type 4 Infections among Children with Acute Respiratory Infections during the 2011–2012 Winter Season in Yamagata, Japan

Chieko Abiko¹, Katsumi Mizuta^{1*}, Yoko Aoki¹, Tatsuya Ikeda¹,
Tsutomu Itagaki², Masahiro Noda³, Hirokazu Kimura³, and Tadayuki Ahiko¹

¹Department of Microbiology, Yamagata Prefectural Institute of Public Health, Yamagata 990-0031;

²Yamanobe Pediatric Clinic, Yamagata 990-0301; and

³Infectious Disease Surveillance Center, National Institute of Infectious Diseases, Tokyo 208-0011, Japan

Communicated by Makoto Takeda

(Accepted November 8, 2012)

Human parainfluenza virus type 4 (HPIV4) belongs to genus *Rubulavirus*, subfamily *Paramyxovirinae* of family *Paramyxoviridae* (1). Although serological surveys indicate that infection may be common, and even universal, reports and descriptions of HPIV4-associated diseases are limited (1–4). Thus, although mild and severe respiratory illness associated with HPIV4 infections are reported, correlations between HPIV4 infections and specific clinical syndromes, age of the child, and time of the year, or seasonal patterns have not been established thus far (1–8). Due to the difficulties in isolating HPIV4, such as slow growth, trypsin concentration, and subtle cytopathic effect (4), its diagnosis has probably been underestimated in the past. However, even though previously undetected cases can now be identified with more sensitive methods such as real-time reverse transcription-PCR (RT-PCR) (1,2), the number of HPIV4 infections reported is still quite low. For example, only 88 cases, including our 28 cases from Yamagata in 2011, were reported between 1982 and 2011 in Japan, according to the National Epidemiologi-

cal Surveillance of Infectious Diseases (NESID) system (9). In this study, we describe an outbreak of HPIV4 infections during the 2011–2012 winter season in Yamagata, Japan.

We carried out a longitudinal epidemiological study of acute respiratory infections (ARIs) among children in Yamagata, Japan, primarily based on virus isolation using a microplate method (10). Six cell lines, namely HEF, HEp-2, Vero or VeroE6, MDCK, RD-18S, and GMK, have been used since 2001. In 2008, we added the HMV-II cell line, primarily for the isolation of HPIVs (10–12). Then, when we accidentally succeeded in isolating one HPIV4 strain from a nasopharyngeal sample collected in October 2011, we added the LLC-MK2 cell line, which is reportedly sensitive for the isolation of HPIV4 (13). Further, we performed RT-PCR screening for HPIV4 in fresh and stocked frozen samples. In the latter effort, cases found to be positive using the influenza virus test kit were excluded. Finally, we included 1,499 nasopharyngeal swab specimens obtained from patients with ARIs at pediatric clinics between January

Table 1. Primers used to amplify and sequence the HN region to differentiate between HPIV4a and HPIV4b

Primer	Sequence (5'–3')	Position (AB543336)
Para4-HN-F1-710	CAACAATCCAGARRGACGTCACA	7409–7431
Para4-HN-F2-711	AGATTAYCCCAATTTATTCCAACCTG	7995–8220
Para4-HN-F3-712	AGGAGCAAAAGAYTCATACACAATAACT	8583–8610
Para4-HN-R2-715	CATTCTTTTGGRCAAGCCTTA	8930–8910
Para4-HN-R3-717	GGTTCCGCTTGATGGACTGT	9508–9489
Para4-HN-R4-718	GGCCGATGGTCCTTCGTATA	9567–9548
Para4-HN-F4-719	CCATCATTYTCCYTARGTCAAA	8049–8071
Para4-HN-F5-720	TGCAGAGGGTCGMYTATATAAYATTG	8721–8746
Para4-HN-R3-721	CGATTCTCCRTTCATTTAAAT	8228–8207
Para4-HN-R5-723	TGATTCTATADAGGCTYGGATADGG	8812–8788
		Position (AB543337)
Para4-HN-R1-714	TTTGTTATTGATGGGTGATGTTTCAC	9467–9443

*Corresponding author: Mailing address: Department of Microbiology, Yamagata Prefectural Institute of Public Health, Tokamachi 1-6-6, Yamagata 990-0031, Japan. Tel: +81-23-627-1373, Fax: +81-23-641-7486, E-mail: mizutak@pref.yamagata.jp

2011 and April 2012. We screened the polymerase L gene coding region using the previously reported AVU-RUB-F1, AVU-RUB-F2, and AVU-RUB-R primers (14), and we further amplified the hemagglutinin-neuraminidase (HN) glycoprotein region in HPIV4-positive specimens to differentiate between HPIV4a and HPIV4b using our original primers shown in Table 1. We registered sequence data with GenBank (accession nos. AB753462–AB753479). When we found syncytia formation on the HMV-II and/or LLC-MK2 cell lines, we also carried out immunofluorescent staining to identify HPIV4, using two commercially available antibodies: mouse anti-parainfluenza 4 MAB8780 (Millipore, Temecula, Calif., USA) and FITC-conjugated rabbit anti-mouse immunoglobulins (DAKO, Glostrup, Denmark).

Among the 1,499 clinical specimens, all 43 HPIV4-positive cases were detected by RT-PCR, and the virus was isolated on the HMV-II and/or LLC-MK2 cell lines in 12 of the 43 cases (Fig. 1). The monthly distribution of HPIV4 detection during the study period is shown in Fig. 2. Further, 37 (86%) HPIV4-positive cases were found between September 2011 and February 2012. In other months, only 0–1 cases were detected. Sequence data were analyzed with CLUSTAL W version 1.83, and a phylogenetic tree was constructed via the

neighbor-joining method (Fig. 3). Among the 43 HPIV4-positive cases, 7 were identified as HPIV4a, 11 as HPIV4b, while the others remained unidentified because the HN region was not amplified enough for the analysis.

Among the 43 HPIV4-positive cases, 4 patients (9.3%) were aged <1 years, 10 (23.2%) were aged 1 year, 2 (4.7%) were aged 2 years, 3 (7%) were aged 3 years, 5 (11.6%) were aged 4 years, 6 (14%) were aged 5 years, 6 (14%) were aged 6–9 years, and 7 (16.2%) were aged 10–15 years. We detected plural viruses, including rhinovirus, human metapneumovirus, enterovirus, cytomegalovirus, and adenovirus in 11 cases. Among the 32 HPIV4-only cases, 25 (78.1%) were diagnosed as nasopharyngitis, 5 (15.6%) as bronchitis, and others as laryngitis and viral exanthema.

A number of HPIV1–3 strains have been isolated with our surveillance system using the microplate method (12); however, HPIV4 strains have been rarely isolated. A local outbreak of HPIV4 was observed for the first time during the winter season of 2011–2012, as described here. Since the number of reported cases of HPIV4 in Japan is quite limited (9), few HPIV4-associated outbreaks have been observed apart from that presented by Watanabe et al. (13). Even worldwide,

Fig. 1. Cytopathic effect and immunofluorescent staining of HPIV4 strains isolated from children with acute respiratory infection. Syncytia formation was observed on LLC-MK2 cell line (A) and on HMV-II cell line (B). The HPIV4-inoculated cells were stained using the indirect immunofluorescent method (C).

Fig. 3. Phylogenetic tree for the partial (1599 bp) sequence of the HN regions of HPIV4 strains detected in Yamagata, Japan between 2011 and 2012 as well as for the reference strains (GenBank accession numbers are shown). The numbers near the branches are the bootstrap probabilities (%).

Fig. 2. Monthly distribution of acute respiratory infections associated with human parainfluenza virus type 4 (HPIV4) in children between January 2011 and April 2012 in Yamagata, Japan.

the number of HPIV4 cases reported is quite limited. Laurichesse et al. and Fry et al. reported that the number of HPIV4-positive cases was markedly smaller than that of HPIV1–3-positive cases (5,15). Lau et al. reported 41 HPIV4 cases during an outbreak in a developmental disability unit and 35 cases (1.2%) among 2,912 nasopharyngeal aspirates in a 1-year study (16,17); Ren et al. described 25 (10.2%) HPIV4 cases among 246 HPIV-positive cases over a 3-year period (6). Finally, Scott et al. described 13 HPIV4-positive cases, most of which were severe cases, during their 6-year study (7). Thus, our study experience together with the limited data, indicates the difficulties associated with the isolation and detection of HPIV4. To clarify the etiology and epidemiology of HPIV4 infections, HPIV4 infections should be recognized as common diseases that require active surveillance using sensitive cell lines such as LLC-MK2, as well as combining molecular method such as RT-PCR.

Watanabe et al. conducted an epidemiological study of HPIV4 among children with ARIs in Sendai and Yamagata. They isolated HPIV4 from 129 (0.8%) of 16,187 clinical specimens using the LLC-MK2 cell line between 2003 and 2009, and they commonly isolated HPIV4 during autumn (13). Based on their study, we believe that a number of HPIV4 infections in Yamagata were missed, especially those during the autumn season. Vachon et al. suggested that HPIV4 infections can be relatively common during the autumn and winter seasons (18); Billaud et al. reported that they detected 18 of 20 HPIV4-positive cases during the autumn and winter (8). Now that we have succeeded in isolating and detecting HPIV4, we hope to determine if HPIV4 outbreaks commonly occur during autumn. Watanabe et al. also stated that approximately two-thirds of HPIV4-positive patients were diagnosed with lower respiratory infections such as pneumonia, bronchiolitis, and bronchitis (13). However, we found only 5 cases of bronchitis, and most of the patients in our study were diagnosed with nasopharyngitis. Thus, the clinical picture of HPIV4 remains controversial.

In conclusion, epidemiological data on HPIV4 are still quite limited and further studies are needed to clarify the etiology and epidemiology of HPIV4 infections.

Acknowledgments We thank the medical staff and people of Yamagata Prefecture for their collaboration in specimen collection for the surveillance of viral infectious diseases.

This work was supported by a grant-in-aid from the Japan Society for the Promotion of Science and for Research on Emerging and Re-emerging Infectious Diseases of the Ministry of Health, Labour and Welfare, Japan.

Conflict of interest None to declare.

REFERENCES

- Psarras, S., Papadopoulos, N.G. and Johnston, S.L. (2009): Parainfluenza viruses. p. 409–439. In A.J. Zuckerman, J.E. Banatvala, B.D. Schoub, et al. (ed.), *Principle & Practice of Clinical Virology*. Wiley-Blackwell, West Sussex, United Kingdom.
- Glezen, W.P. and Denny, F.W. (1997): Parainfluenza viruses. p. 551–567. In A.S. Evans and R.A. Kaslow (ed.), *Viral Infections of Humans*. Plenum Medical Book Company, New York.
- Boyer, K.M. (2009): Non bacterial pneumonia. p. 288–301. In R.D. Feigin, J.D. Cherry, G.J. Demmler-Harrison, et al. (ed), *Textbook of Pediatric Infectious Diseases*. 6th ed. Saunders Elsevier, Philadelphia.
- Robinson, C.C. (2009): Respiratory viruses. p. 203–248. In S. Specter, R.L. Hodinka, S.A. Young, et al. (ed), *Clinical Virology Manual*. 4th ed. ASM press, Washington, D.C.
- Laurichesse, H., Dedman, D., Watson, J.M., et al. (1999): Epidemiological features of parainfluenza virus infections: laboratory surveillance in England and Wales, 1975–1977. *Eur. J. Epidemiol.*, 15, 475–484.
- Ren, L., Gonzalez, R., Xie, Z., et al. (2011): Human parainfluenza virus type 4 infection in Chinese children with lower respiratory tract infections: a comparison study. *J. Clin. Virol.*, 51, 209–212.
- Scott, L., Asha, D., Allison, I., et al. (1997): Parainfluenza virus type 4 infections in pediatric patients. *Pediatr. Infect. Dis. J.*, 16, 34–38.
- Billaud, G., Morfin, F., Vabret, A., et al. (2005): Human parainfluenza virus type 4 infections: a report of 20 cases from 1998 to 2002. *J. Clin. Virol.*, 34, 48–51.
- National Epidemiological Surveillance of Infectious Diseases (NESID) system. Online at <https://nesid3g.wish.mhlw.hq.admix.go.jp/GKWeb/GKMainServlet?action_id=GKMainMenu>. Accessed 27 June 2012.
- Mizuta, K., Abiko, C., Aoki, Y., et al. (2008): Analysis of monthly isolation of respiratory viruses from children by cell culture using a microplate method: a two-year study from 2004 to 2005 in Yamagata, Japan. *Jpn. J. Infect. Dis.*, 61, 196–201.
- Mizuta, K., Saitoh, M., Kobayashi, M., et al. (2011): Detailed genetic analysis of hemagglutinin-neuraminidase glycoprotein gene in human parainfluenza virus type 1 isolates from patients with acute respiratory infection between 2002 and 2009 in Yamagata Prefecture, Japan. *Virol. J.*, 8, 533.
- Mizuta, K., Abiko, C., Aoki, Y., et al. (2012): Epidemiology of parainfluenza virus type 1, 2 and 3 infections based on virus isolation between 2002 and 2011 in Yamagata, Japan. *Microbiol. Immunol.*, 56, 855–858.
- Watanabe, O., Ohmi, A., Nagai, Y., et al. (2011): Abstract 32. 65th Annual Meeting of Tohoku Society of Microbiology. Online at <<http://minfo.id.yamagata-u.ac.jp/Imm/65bact/img/shouroku.pdf>> (in Japanese).
- Tong, S., Chern, S.W., Li, Y., et al. (2008): Sensitive and broadly reactive reverse transcription-PCR assays to detect novel paramyxoviruses. *J. Clin. Microbiol.*, 46, 2652–2658.
- Fry, A.M., Curns, A.T., Harbour, K., et al. (2006): Seasonal trends of human parainfluenza viral infections: United States, 1990–2004. *Clin. Infect. Dis.*, 43, 1016–1022.
- Lau, S.K.P., To, W., Tse, P.W.T., et al. (2005): Human parainfluenza virus type 4 outbreak and the role of diagnostic tests. *J. Clin. Microbiol.*, 43, 4515–4521.
- Lau, S.K.P., Li, K.S.M., Chau, K., et al. (2009): Clinical and molecular epidemiology of human parainfluenza virus 4 infections in Hong Kong: subtype 4B as common as subtype 4A. *J. Clin. Microbiol.*, 47, 1549–1552.
- Vachon, M., Dionne, N., Leblanc, E., et al. (2006): Human parainfluenza type 4 infections, Canada. *Emerg. Infect. Dis.*, 12, 1755–1758.