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Human Parainfluenza Virus 4 Outbreak and the Role of Diagnostic Tests

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Owing to the difficulties in isolating the virus and the lack of routine surveillance, the clinical significance of human parainfluenza virus 4 (HPIV-4) is less well defined than that of the other human parainfluenza viruses. We describe the first outbreak of HPIV-4 infection in a developmental disabilities unit, involving 38 institutionalized children and three staff members, during a 3-week period in autumn 2004. Most subjects had upper respiratory tract infections (URTI), while lower respiratory tract infections (LRTI) occurred in three children (7%), one complicated by respiratory failure requiring ventilation support. All patients recovered. Nasopharyngeal aspirates tested for HPIV-4 were positive by reverse transcriptase PCR (RT-PCR) in all 41 cases (100%), by direct immunofluorescence in 29 of 39 tested cases (74%), and by cell cultures in 6 of 37 cases (16%), and serum was positive for antibodies against HPIV-4 in all 35 cases (100%) with serum samples available. In addition, RT-PCR detected HPIV-4 in four children (three LRTI and one URTI) out of 115 patients with community-acquired respiratory tract infection. Molecular analysis of the 1,198-bp phosphoprotein sequences showed that HPIV-4 isolates among the cases were genetically similar, whereas the community controls were more genetically distant, supporting nosocomial transmission of a single HPIV-4 genotype during the outbreak. Moreover, the HPIV-4 causing the outbreak is more closely related to HPIV-4A than HPIV-4B. HPIV-4 may be an important cause of more severe respiratory illness in children. The present RT-PCR assay is a sensitive, specific, and rapid method for the diagnosing HPIV-4 infection. To better define the epidemiology and clinical spectrum of disease of HPIV-4 infections, HPIV-4 should be included in the routine panels of respiratory virus detection on respiratory specimens.

Human parainfluenza viruses (HPIVs) are common respiratory tract pathogens that can infect persons of any age. They are enveloped, negative-sense RNA viruses that belong to the subfamily *Paramyxovirinae* of the family *Paramyxoviridae*. There are four genetically and antigenically different types, HPIV types 1 to 4 (HPIV-1 to -4) (3). HPIV-1 and HPIV-3 belong to the genus *Respirovirus*, whereas HPIV-2 and HPIV-4 to the genus *Rubulavirus*. HPIV-1, -2, and -3 are major causes of lower respiratory tract infections in infants, young children, and immunocompromised hosts and upper respiratory tract infections in older children and adults (3, 19). In particular, HPIV-1 and HPIV-3 have been identified as important causes of outbreaks of respiratory tract infections, especially in institutional settings (4, 6, 13). In contrast, HPIV-4 has been regarded as less clinically important and associated with milder respiratory illness, and it has not been reported to cause major outbreaks of respiratory tract infections.

HPIV-4 was first isolated in 1959 from a male college student with a mild upper respiratory tract infection (9). The virus can be found worldwide and is thought to be relatively ubiquitous. Serological studies have shown that it may account for as much as 3% of all respiratory tract infections (16) and have demonstrated 50% to 90% seroprevalence in children and young adults (7). However, because of its inherently low recovery rate in cell culture and reported association with mild respiratory disease, HPIV-4 is not included in the routine panels of respiratory virus antigen detection on nasopharyngeal aspirates (NPAs) and other respiratory specimens in most clinical virology laboratories. This may have led to even fewer recognized cases of HPIV-4 infections and less appreciation of its clinical significance. In this report, we describe the first outbreak of HPIV-4 infections, which occurred in children institutionalized in a developmental disabilities unit. Different methods for diagnosis of HPIV-4 infections were also compared, and molecular epidemiology was used to confirm nosocomial transmission in the outbreak.

MATERIALS AND METHODS

Patient population. From 5 November 2004 through 25 November 2004, 38 cases of HPIV-4 infection were identified in children institutionalized in the Developmental Disabilities Unit (DDU) at Caritas Medical Center, a regional hospital in Hong Kong. In additional, three staff members were infected. No cases occurred in the subsequent 4 weeks. The indications for admission to the

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DDU were severe mental handicap with a requirement for long-term residential care.

Setting. The DDU consisted of four wards located in the first to the fourth floors of the same building, with four cubicles (A, B, C, and D) per ward. Approximately 200 children were institutionalized and 120 health care workers were working in the DDU at the time of the outbreak. Most of the residents were immobile and totally dependent on assistance to carry out daily living activities, and many had congenital and multiple medical problems. Recurrent chest infections were common in those that were immobilized, and several cases of febrile illness per week were not unexpected, according to records of a fever surveillance system. During the day, the majority of these children (88%) also attended a special school connected to DDU by corridors. Mixing activities among children from different wards occurred during the classes. Family members often visited the children in the wards.

Epidemiological investigation and infection control measures. After the first five cases of febrile respiratory illness were identified in the DDU, the possibility of an outbreak was considered. Clinical records of all patients in the past 2 weeks were studied and active surveillance carried out to identify patients with symptoms or signs of respiratory tract infections. For those with suggestive clinical features, their age and sex, date of disease onset and duration, clinical manifestations, comorbidities, length of stay in the hospital, dates of attending special school, and daytime and hospital room locations were also recorded. Multimodal infection control measures were implemented, with isolation of suspected cases. Isolation rooms were equipped with dedicated medical equipment, which was cleaned thoroughly between patients. Health care workers were required to practice stringent droplet and contact precautions, including hand washing before and after all routine patient contact.

Clinical specimens and microbiological investigations. Sputum samples, NPAs, and blood samples were collected from patients with respiratory symptoms during the study period. Gram staining and bacterial cultures were performed on sputum specimens. The NPAs were assessed by direct antigen detection for influenza A and B viruses, parainfluenza viruses 1, 2, and 3, respiratory syncytial virus, and adenovirus by immunofluorescence (22) and for influenza A, B, and C viruses, human metapneumovirus, rhinovirus, human coronaviruses, and severe acute respiratory syndrome coronavirus (SARS-CoV) by RT-PCR (14, 15, 18). Serological assays for antibodies against *Mycoplasma*, *Chlamydia*, *Legionella*, and SARS-CoV included SERODIA-MYCO II (Fujirebio Inc., Tokyo, Japan), *Chlamydia pneumoniae*-specific immunoglobulin G (IgG) microimmunofluorescence assay (Focus Technologies, Cypress, CA), indirect immunofluorescence assay (MRL, San Diego, CA), and a recently developed enzyme-linked immunosorbent assay, respectively (20, 21).

RT-PCR, viral isolation, direct immunofluorescence, and antibody detection for HPIV-4. Since initial investigations for respiratory pathogens were all negative, reverse transcriptase PCR (RT-PCR) for HPIV-4 was performed on NPAs collected from symptomatic patients. One hundred fifteen NPAs from patients with community-acquired respiratory tract infections sent to the microbiology laboratory of Queen Mary Hospital were also subjected to RNA extraction and RT-PCR as controls. Viral RNA was extracted directly from the NPA specimens using a QIAamp viral RNA minikit (Qiagen, Hilden, Germany). The RNA pellet was resuspended in 10 μl of DNase-free, RNase-free, double-distilled water and was used as the template for RT-PCR. A 246-bp fragment of the phosphoprotein gene of HPIV-4 was amplified by RT-PCR using primers (LPW 1778 5′-AAA GAATTAGGTGCAACCAGTC-3′ and LPW 1779 5′-GTGTCTGATCCCATA AGCAGC-3′) modified from a previously published protocol (1). For those NPAs positive for HPIV-4 by RT-PCR with an adequate amount of RNA, the complete phosphoprotein genes were amplified and sequenced using additional primers designed by multiple alignment of the phosphoprotein genes of HPIV-4 available in GenBank. The RNA was converted to cDNA by a combined random-priming strategy using a SuperScript II kit (Invitrogen, San Diego, CA). The PCR mixture (50 μl) contained cDNA, PCR buffer (10 mM Tris-HCl [pH 8.3], 50 mM KCl, 3 mM $MgCl_2$ and 0.01% gelatin), 200 μM (each) deoxynucleoside triphosphates, and 1.0 U *Taq* polymerase (Boehringer Mannheim, Germany). The mixtures were amplified in 40 cycles of 94°C for 1 min, 48°C for 1 min, and 72°C for 1 min and a final extension at 72°C for 10 min in an automated thermal cycler (Perkin-Elmer Cetus, Gouda, The Netherlands). The PCR products were gel purified using a QIAquick gel extraction kit (Qiagen, Hilden, Germany). Both strands of the PCR products were sequenced twice with an ABI Prism 3700 DNA analyzer (Applied Biosystems, Foster City, CA), using the PCR primers.

After it was determined that some of the NPAs were RT-PCR positive for HPIV-4, NPAs which had been obtained in sufficient amounts were inoculated into Vero E6 (African green monkey kidney) cells and/or LCC-MK2 (rhesus monkey kidney) cells, in the presence of trypsin (1 μg/ml). Infected Vero E6 cells were identified by immunofluorescence, using specific monoclonal antibodies to HPIV-4 (Chemicon International, Temecula, CA). Infected LCC-MK2 cells were identified by terminal hemadsorption on day 10. Direct immunofluorescence for HPIV-4 antigen detection was also performed on NPAs from cases with sufficient cells, according to the manufacturer's instructions (Chemicon International, Temecula, CA). Detection of antibody against HPIV-4 was performed on paired serum samples from outbreak cases using indirect immunofluorescence on HPIV-4 culture lysates as modified from a previously published protocol (14).

HPIV-4 infections during the outbreak period were defined as having both acute onset of fever and symptoms of respiratory tract infections, as well as laboratory evidence of HPIV-4 infection, which includes one or more of the following: (i) NPA positive for HPIV-4 by culture, (ii) NPA positive for HPIV-4 RNA by RT-PCR, (iii) NPA positive for HPIV-4 antigen by direct immunofluorescence, and (iv) a ≥4-fold rise in HPIV-4 IgG titer or a single HPIV-4 IgG titer of ≥1:400 in serum samples.

Molecular analysis. The nucleotide and deduced amino acid sequences of the PCR products from 35 cases and four community controls were compared with known phosphoprotein sequences of members of the *Paramyxoviridae* in the GenBank database by multiple sequence alignment using the ClustalW program (17). The phylogenetic relationships were determined using ClustalX version 1.81 (8) and the neighbor-joining method with GrowTree (Genetics Computer Group, Inc.). A total of 1,198 nucleotide or 399 amino acid positions were included in the analysis.

RESULTS

HPIV-4 outbreak characteristics. The characteristics of cases with HPIV-4 infection are summarized in Table 1 and Fig. 1. Thirty-eight children from the DDU (cases 1 to 38) met the case definition for HPIV-4 infection in the outbreak. In addition, a staff from the special school (case 39) and two hospital health care workers (cases 40 and 41) were infected. Of the total 41 cases, 22 were males and 19 were females. The median age was 11 (range, 4 to 46). Thirty-one of the 38 infected children were from the ward at the first floor and the other seven from another ward at the third floor. No cases were identified from the other two wards at the second and fourth floors. Apart from the three adult cases, all cases had underlying diseases. The number of cases peaked at day 6. In response to the outbreak, the infection control measures stated above were verified and reinforced by the infection control team. This was immediately followed by a decline in the number of cases and termination of the outbreak subsequently.

Clinical manifestations. All 41 patients had symptoms attributed to HPIV-4 infection. Fever and cough with or without sputum were the most common symptoms, followed by coryza, sore throat, and vomiting. The infection typically began with high fever of 39°C to 40°C which lasted for 2 to 5 days, followed by defervescence. Three patients had evidence of lower respiratory tract infection, while the other 38 patients had symptoms and signs localized to the upper respiratory tract. All three patients with lower respiratory tract infection were children from the first floor. None of the patients died, although the infection was complicated by gastrointestinal bleeding in case 15 and respiratory failure, rhabdomyolysis, and acute renal failure in case 19, who required intensive care and mechanical ventilation. All patients recovered eventually.

Microbiological investigations. Apart from the isolation of a strain of *Streptococcus pneumoniae* from the sputum of the index case (case 1), which is likely due to a secondary bacterial infection, all sputum cultures from other cases were negative for respiratory pathogens. All NPAs for direct antigen detection of respiratory viruses and RT-PCR for influenza A, B, and

TABLE 1. Summary of clinical characteristics and HPIV-4 investigation results for the 41 outbreak cases[a]

Case	Sex/age (yr)	Ward/cubicle	Onset day from outbreak	Underlying disease	Infection	CXR findings	Result of:				Titer of IgG against HPIV-4	
							RT-PCR	Direct IF	Vero E6 culture	LCC-MK2 culture	Acute phase	Convalescent phase
1	M/7	1/D	1	Mental retardation of unknown cause, bronchiectasis, recurrent chest infections, chair bound	URTI	No new infiltrates	+	+	NA	+	1:1,600	1:800
2	F/6	1/D	3	Hypoxic ischemic encephalopathy, bronchiectasis, recurrent chest infections, chair bound	URTI	No new infiltrates	+	+	−	−	1:12,800	1:6,400
3	M/8	1/C	3	Birth asphyxia, recurrent chest infections, chair bound	LRTI	Bilateral infiltrates	+	+	−	+	1:100	>1:400
4	M/15	1/B	3	Lissencephaly, bronchiectasis, chair bound	URTI	No new infiltrates	+	−	−	−	1:400	1:1,600
5	F/7	3/A	3	Subarachnoid hemorrhage, chair bound	URTI	Clear	+	−	−	−	1:400	1:800
6	F/8	1/B	4	Congenital myopathy, chair bound	URTI	Clear	+	−	−	−	1:1,600	1:3,200
7	M/10	1/B	5	Birth asphyxia, chair bound	URTI	Clear	+	+	−	+	1:100	>1:400
8	M/10	1/B	5	Inborn errors of metabolism, recurrent chest infections, chair bound	URTI	Clear	+	+	−	−	1:100	>1:400
9	M/8	1/C	5	Congenital myotonic dystrophy, chair bound	URTI	Clear	+	+	−	−	1:25	>1:400
10	M/16	1/A	5	X-linked mental retardation, bronchiectasis, recurrent chest infections, chair bound	URTI	No new infiltrates	+	−	−	−	1:12,800	1:6,400
11	F/7	1/C	6	Mental retardation of unknown cause, bronchiectasis, recurrent chest infections, chair bound	URTI	Clear	+	+	+	+	1:100	>1:400
12	M/10	1/A	6	Acute or chronic subdural hematoma, recurrent chest infections, chair bound	URTI	No new infiltrates	+	+	NA	−	NA	NA
13	F/11	3/A	6	Birth asphyxia	URTI	Clear	+	−	−	−	1:200	1:400
14	F/9	3/C	6	Isolated sulfite oxidase deficiency, chair bound	URTI	No new infiltrates	+	+	+	−	<1:25	>1:400
15	M/4	1/C	6	Congenital cytomegalovirus infection, recurrent chest infections, chair bound	URTI	Clear	+	+	−	+	<1:25	1:400
16	M/11	3/A	6	Lennox-Gastaut syndrome	URTI	Clear	+	+	−	−	1:100	1:400
17	M/12	1/C	7	Encephalitis, recurrent chest infections, chair bound	URTI	Clear	+	+	−	−	1:100	>1:400
18	F/8	1/C	7	Chromosomal abnormalities, cyanotic heart disease, recurrent chest infections, chair bound	URTI	Clear	+	+	−	+	<1:25	1:400
19	M/16	1/B	8	Birth asphyxia, recurrent chest infections, chair bound	LRTI	Right lung haziness	+	−	−	−	1:3,200	1:3,200
20	M/17	1/B	8	Birth asphyxia, chair bound	URTI	Not taken	+	+	−	+	1:25	>1:400
21	F/13	1/B	8	Birth asphyxia, bronchiectasis, recurrent chest infections, chair bound	URTI	No new infiltrates	+	−	+	+	1:25	>1:400
22	M/15	1/C	8	Ornithine transcarbamylase deficiency, recurrent chest infections. chair bound	URTI	Clear	+	+	−	−	1:25	>1:400
23	M/10	1/B	9	Prematurity, chair bound	URTI	Not taken	+	−	+	−	1:200	1:800
24	F/11	1/A	9	Mental retardation of unknown cause, chair bound	URTI	No new infiltrates	+	+	−	+	<1:25	>1:400
25	F/12	1/A	10	Cri-du-chat syndrome, Eisenmenger syndrome, chair bound	URTI	Not taken	+	+	+	−	<1:25	>1:400
26	M/15	3/D	10	Lissencephaly, chair bound	URTI	Clear	+	−	−	−	<1:25	>1:400
27	F/15	1/C	10	Early infantile epileptic encephalopathy, recurrent chest infections. chair bound	LRTI	Right lower zone consolidation	+	+	−		1:100	>1:400
28	M/6	1/B	11	Inborn errors of metabolism, recurrent chest infections, chair bound	URTI	No new infiltrates	+	+	+	+	1:25	>1:400
29	F/13	3/C	11	Neonatal jaundice, chair bound	URTI	Clear	+	+	−	+	1:25	>1:400
30	M/14	1/C	12	Arthrogryposis multiplex congenita, chair bound	URTI	Not taken	+	+	−	+	1:25	>1:400
31	M/13	1/B	12	Mental retardation of unknown cause, recurrent chest infections, chair bound	URTI	No new infiltrates	+	+	−	+	1:100	>1:400

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TABLE 1—*Continued*

Case	Sex/age (yr)	Ward/cubicle	Onset day from outbreak	Underlying disease	Infection	CXR findings	Result of:				Titer of IgG against HPIV-4	
							RT-PCR	Direct IF	Vero E6 culture	LCC-MK2 culture	Acute phase	Convalescent phase
32	F/7	3/C	13	Hypoxic ischemic encephalopathy, chair bound	URTI	Clear	+	+	−	+	<1:25	>1:400
33	F/15	1/B	14	Mental retardation of unknown cause, recurrent chest infections, chair bound	URTI	Clear	+	+	−	−	NA	NA
34	M/10	1/C	14	Status epilepticus, chair bound	URTI	Clear	+	+	−	−	1:25	>1:400
35	F/13	1/C	16	Birth asphyxia, recurrent chest infections, chair bound	URTI	Clear	+	+	−	−	1:100	>1:400
36	M/11	1/C	16	Congenital cytomegalovirus infection, recurrent chest infections, chair bound	URTI	Clear	+	+	−	−	1:400	>1:1,600
37	M/12	1/B	16	Mental retardation of unknown cause, chair bound	URTI	Clear	+	+	−	−	1:100	1:400
38	F/15	1/B	21	Rubenstein-Taybi syndrome, chair bound	URTI	Clear	+	+	−	−	NA	NA
39	F/25		8	None	URTI	Clear	+	−	−	NA	NA	NA
40	F/46		17	None	URTI	Not taken	+	NA	NA	NA	NA	NA
41	F/25		17	None	URTI	Clear	+	NA	NA	NA	NA	NA

[a] URTI, upper respiratory tract infection; LRTI, lower respiratory tract infection; CXR, chest radiograph; IF, immunofluorescence; NA, not available.

C viruses, human metapneumovirus, rhinovirus, human coronaviruses, and SARS-CoV were negative. All sera for antibodies against *Mycoplasma*, *Chlamydia*, *Legionella*, and SARS-CoV were also negative. Results of investigations for HPIV-4 are summarized in Table 1. RT-PCR of the phosphoprotein gene from the NPAs of all 41 cases (100%) showed bands of about 250 bp. Direct immunofluorescence for HPIV-4 antigen was positive in 29 of 39 tested cases (74%), demonstrating intense cytoplasmic fluorescence in infected cells. Vero E6 cultures for HPIV-4 detected by immunofluorescence were positive in six of 37 cases (16%), whereas LCC-MK2 cultures for HPIV-4 detected by hemadsorption were positive in 14 of 38 cases (37%). Only three cases were positive for HPIV-4 in both culture systems. No cytopathic effect was observed in Vero E6 cultures while syncytial formation was found in a few positive LCC-MK2 cultures. Of the 35 cases with serum samples available, all (100%) demonstrated the presence of antibodies against HPIV-4. A ≥4-fold rise in IgG against HPIV-4 was shown in 28 cases, while a single IgG titer of ≥1:400 was present in the other seven cases (cases 1, 2, 5, 6, 10, 13, and 19). Serum samples were not available for six patients because permission to obtain blood samples was refused by patients or their guardians. In contrast, 4 of the 115 community controls were positive for HPIV-4 by RT-PCR.

Molecular characterization. Sequencing of the complete phosphoprotein genes from the index case sample showed the highest homology to the corresponding sequences of HPIV-4A from GenBank, with 94.5% amino acid and 95.7% nucleotide

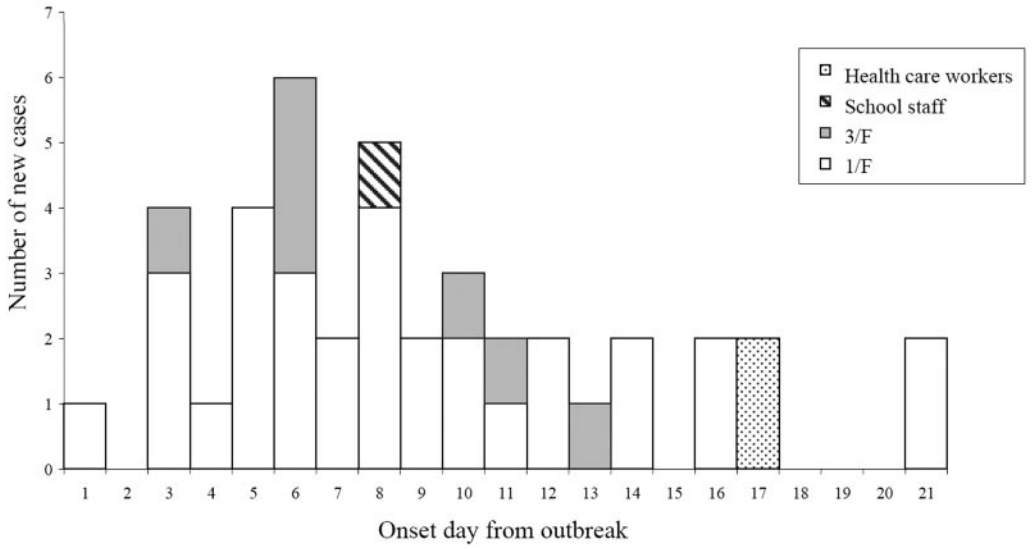

FIG. 1. Epidemic curve summarizing the number of new cases versus day from outbreak. 3/F, cases from the third floor; 1/F, cases from the first floor.

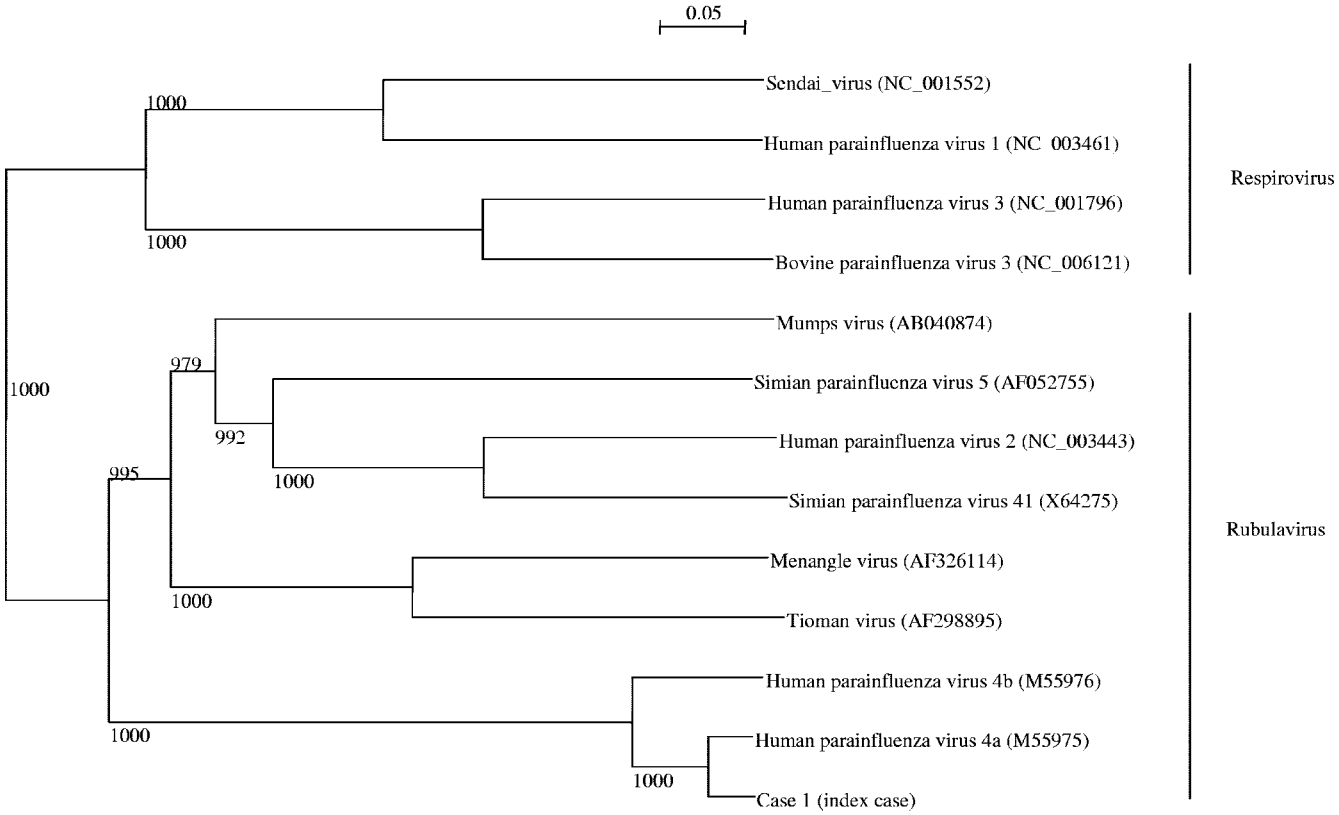

FIG. 2. Phylogenetic analysis of the deduced amino acid sequences of phosphoprotein genes of HPIV-4 from the index case (case 1) in relation to members of the family *Paramyxoviridae*. The tree was constructed by the neighbor-joining method using the Jukes-Cantor correction and bootstrap values calculated from 1,000 trees. The scale bar indicates the estimated number of substitutions per 100 amino acids.

identities (Fig. 2). Sequences from the 36 cases with complete phosphoprotein genes differed by 0 to 2 nucleotides, whereas sequences from the community controls were more genetically distant to the cases, with 4 to 6 nucleotide differences (Fig. 3).

Epidemiologic analysis. An intensive investigation of transmission patterns was carried out during the outbreak. However, no consistent pattern of exposure with regard to health care worker exposure or attendance at day school rooms was identified. The index patient had been frequently visited by her mother, who did not notice any respiratory symptoms throughout.

DISCUSSION

We present the first report of an outbreak of respiratory tract infections associated with HPIV-4. Owing to the difficulties in isolating HPIV-4 and the general unawareness of its clinical significance, the prevalence of HPIV-4 may have been underestimated. HPIV-4 virus is the most difficult HPIV to grow in tissue culture, with late hemadsorption and cytopathic effect, and therefore is only rarely isolated. Although a fluorescein isothiocyanate-conjugated monoclonal antibody directed to HPIV-4 can be used for identification of isolates in tissue cultures and direct detection on respiratory specimens by immunofluorescence assay (16), it is not routinely used in most clinical virology laboratories, which usually include only HPIV-1, -2, and -3 in their routine panels of respiratory virus detection. As a result, HPIV-4 infections are far less readily recognized than HPIV-1, -2, and -3 infections. More than 40 years after its discovery, only a few individual cases or case series of HPIV-4 infection have been described, and these relied mostly on culture results (5, 12, 16). Recently, an RT-PCR assay has been developed for direct detection of HPIV-4 in NPAs from pediatric patients with lower respiratory tract illness and was shown to be more sensitive than cell culture isolation (1). Moreover, the same study also found that HPIV-4 was more frequently detected than HPIV-2 by RT-PCR, supporting the idea that HPIV-4 has been underestimated as a lower respiratory tract pathogen previously. In this study, we demonstrated that HPIV-4 was the cause of an outbreak of respiratory infections in institutionalized pediatric patients. Initial routine investigations for respiratory pathogens were negative. HPIV-4 was found to be the etiological agent only after the RT-PCR assay. Furthermore, by screening 115 NPAs from patients with community-acquired respiratory tract infection using RT-PCR, four patients with HPIV-4 infection were identified, which would not have been recognized in our routine antigen detection tests for respiratory viruses. To better define its clinical importance and seasonal patterns, HPIV-4 should be included in the routine panels of respiratory virus detection on respiratory specimens.

Although HPIV-4 was first recognized to cause relatively mild upper respiratory tract infections in both children and adults, the relative importance of HPIV-4 in causing upper and

FIG. 3. Phylogenetic analysis of phosphoprotein genes of HPIV-4 from 36 cases and four community controls positive for HPIV-4 by RT-PCR. The tree was constructed by the neighbor-joining method using the Jukes-Cantor correction and bootstrap values calculated from 1,000 trees. The scale bar indicates the estimated number of substitutions per 1,000 nucleotides.

lower respiratory tract infections remains to be determined. It has been suggested by some case series that HPIV-4 may be associated with more severe disease. In a report on a series of 10 children with HPIV-4 infection during a 4-year period, five cases were associated with bronchiolitis or pneumonia and one with aseptic meningitis. Eight of the 10 children required hospitalization (16). In another 5-year retrospective study on 13 pediatric patients with HPIV-4 infection, 8 had lower respiratory tract infections, 1 had aseptic meningitis, and 10 required hospitalization (12). It was concluded that HPIV-4 infection may be more common and severe than was previously thought. However, the identification of cases in these studies was based on viral culture results, which may have been dependent on the inoculating viral load and, in turn, the severity of illness. Our report therefore represents the first identification of a cohort of HPIV-4 infections based on more sensitive diagnostic assays. In the present outbreak, 38 of around 200 children and 2 of around 120 hospital health care workers from the DDU were infected. The majority of the cases had mild upper respiratory tract infections. Three children (7%) had lower respiratory tract infections. As for the four community controls, all were children and were hospitalized for acute respiratory illness. Three had lower respiratory tract infections and one had an upper respiratory tract infection. Our results support the idea that HPIV-4 may be an important cause of more severe respiratory illness in children than was previously thought. Recognition of more cases of HPIV-4 would help delineate its epidemiology and clinical spectrum of disease.

The current RT-PCR assay is a sensitive, specific, and rapid method for detecting HPIV-4 infection. In the present study, all 35 patients with available serum samples had specific antibody responses against HPIV-4, with 28 demonstrating a fourfold rise in IgG while the remaining seven had at least one titer of $\geq$1:400. A fourfold rise in IgG was not observed in the latter seven patients, who presented in the early phase of the outbreak, probably because their first serum samples were collected late in the course of their illness and therefore demonstrated high acute-phase IgG titers. All seven patients also had NPAs positive for HPIV-4 by RT-PCR, supporting the idea that they had recent HPIV-4 infections. Although serological testing by indirect immunofluorescence was a sensitive method for diagnosis of HPIV-4 infection, a specific antibody response develops only during the convalescent phase of the illness. Therefore, rapid diagnosis of HPIV-4 infection would rely on viral detection such as RT-PCR or direct immunofluorescence. In a previous study that developed a RT-PCR assay for detection of HPIV-4, it was shown that RT-PCR was more sensitive than culture from NPAs (1). In the same study, a multiplex RT-PCR assay for the simultaneous detection of HPIV-1, -2, -3, and -4 was also developed. In this study, using case definition as the "gold standard," RT-PCR was 100% sensitive for detection of HPIV-4, compared to 74% for direct immunoflu-

orescence assay and 16% to 37% for cell cultures. The recovery rate in cell cultures was particularly low, which is in line with reports from previous researchers. The current RT-PCR was also very specific, as only 4 of the 115 community control NPAs were positive and all four showed distinct phosphoprotein sequences from outbreak cases, suggesting no false-positive cases. As molecular methods become better developed and more widely available in clinical virology laboratories, RT-PCR may be put into routine use for detection of parainfluenza viruses, replacing the immunofluorescence methods.

Molecular analysis suggested that the present HPIV-4 outbreak was caused by a single genotype. Molecular analysis, especially gene sequence analysis, has been conducted in the investigations of outbreaks due to other parainfluenza viruses and was shown to be of great value in understanding epidemiology and transmission cycles (4, 13). The phosphoprotein sequences obtained from 36 outbreak cases differed by no more than two nucleotides, while those from community controls were more genetically diverse. This is in keeping with nosocomial transmission of a single HPIV-4 genotype during the outbreak. Molecular analysis will continue to be a useful tool for studying the epidemiology of viral infections.

The HPIV-4 causing the present outbreak is more closely related to HPIV-4A than HPIV-4B, based on the phosphoprotein gene sequences. HPIV-4 was subdivided into two subtypes, 4A and 4B, based on hemagglutination inhibition and neutralizing antibody tests in 1964 (2, 10). Subsequently, a significant difference has also been demonstrated between the phosphoprotein genes of HPIV-4A and HPIVB (11). While subtyping was not performed in most previously reported case series of HPIV-4 infection because monoclonal antibodies directed against both HPIV-4A and HPIV-4B were used, a study on the development of RT-PCR for detection of HPIV-4 in clinical samples from Spain found that all 10 of their HPIV-4 isolates were subtyped as HPIV-4A with their subtype-specific primers (1). In the present study, all 36 phosphoprotein gene sequences available from the outbreak cases and the four community controls were phylogenetically closer to HPIV-4A than HPIV-4B. Further studies are required to determine the relative importance of HPIV-4A and HPIV-4B in our locality.

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