

Epidemiology of Human Parechovirus Type 3 Upsurge in 2 Hospitals, Freiburg, Germany, 2018

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In 2018, a cluster of pediatric human parechovirus (HPeV) infections in 2 neighboring German hospitals was detected. Viral protein 1 sequence analysis demonstrated co-circulation of different HPeV-3 sublineages and of HPeV-1 and -5 strains, thereby excluding a nosocomial outbreak. Our findings underline the need for HPeV diagnostics and sequence analysis for outbreak investigations.

Most human parechovirus (HPeV) infections cause mild upper respiratory tract symptoms or unspecific febrile illnesses. Severe clinical manifestations, such as meningitis/encephalitis, myocarditis, and newborn sepsis, are caused by HPeV type 3 (HPeV-3) and have been described in children <3 months of age (1). Surveillance data show endemic circulation in several countries, such as the Netherlands (2) and the United States (3), but studies have discussed the epidemic potential of HPeV-3 in other countries, including Japan (4), Australia (5), and the United Kingdom (6). Nosocomial transmission has been documented (7).

However, in most outbreak investigations, determination of HPeV types was performed retrospectively (7,8). We report on our investigations on a cluster of HPeV infections in 2 neighboring hospitals in Freiburg, Germany. We provide evidence that rapid phylogenetic analysis can assist in outbreak investigations.

The Study

During routine diagnostic testing of clinical samples from infants and young children in July 2018, we

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detected an increase in HPeV cases (Figure 1). We collected ≥ 1 clinical specimens from most patients (Table 1). During July 9–August 25, 2018, we documented 19 cases, compared with 4 (2016) and 2 (2017) from this same time span, all using the same assays and diagnostic testing algorithm. In September 2018, only 2 patients tested HPeV-positive; no additional cases were identified during October and November 2018. HPeV diagnostic procedures were performed upon the request of the treating physician. For the detection of HPeV, we used commercial multiplex PCR panels: FTD respiratory pathogens 21 (Fast Track Diagnostics [FTD]; Siemens Healthineers, <https://www.siemens-healthineers.com>) for respiratory specimens and FTD EPA for cerebrospinal fluid (CSF), plasma, and fecal samples. Patients were hospitalized on 1 ward in hospital A and 4 wards in hospital B (Appendix Figure, <http://wwwnc.cdc.gov/EID/article/25/7/19-0257-App1.pdf>). We retrieved medical data on HPeV-positive patients from the hospital-based information system. We obtained written informed consent from parents or guardians.

The age of the 2018 HPeV-positive patients ranged from 10 days to 19 months (median 1 month), with 88% of patients being <4 months of age (Table 1). Plasma samples (n = 14) had a diagnostic yield of 100%. The median duration of hospitalization was 4 days (range 3–23 days). The main clinical symptoms of HPeV-3 cases were fever (n = 21; 100%) and poor feeding (n = 16; 76%) (Table 2). None of our patients required admission to a pediatric intensive care unit. All of our patients were discharged from the hospital without complications.

After we detected the first cases in July 2018, we performed molecular typing of HPeV by amplifying and sequencing the complete viral protein 1 (VP1) genomic region (9). Of the 25 HPeV strains detected in Freiburg in 2018, 21 were typed as HPeV-3, 2 were assigned to HPeV-1, and 2 to HPeV-5 (Table 1). This compares with 7 HPeV-1 and 6 HPeV-3 types in Freiburg in 2016, and 3 HPeV-1, 3 HPeV-3, and 5 strains not typed in 2017 (Figure 1).

For phylogenetic analyses, we included HPeV strains detected during January 2016–September 2018 at another 4 university hospitals: Würzburg (n = 56)

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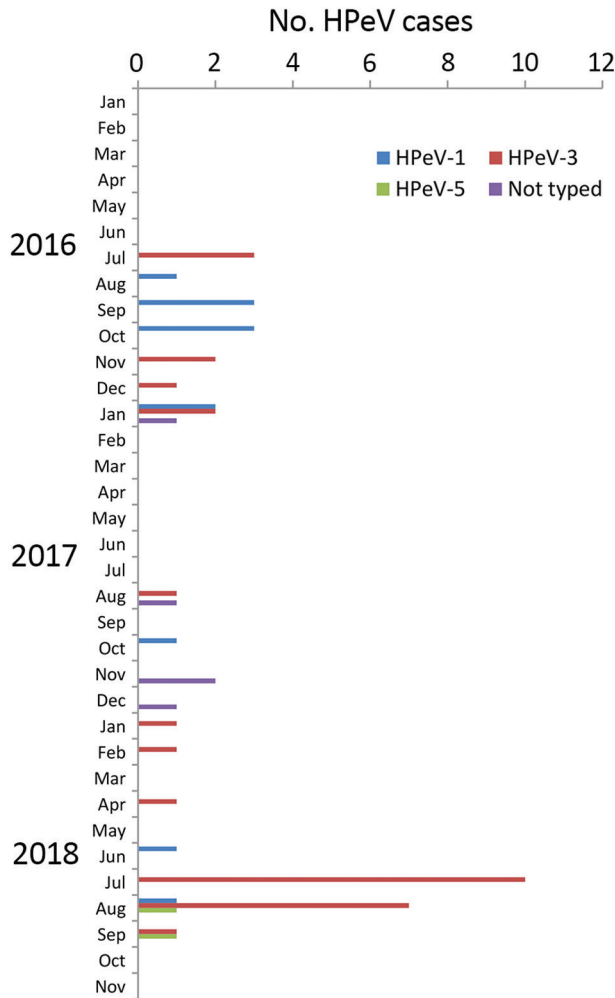


Figure 1. Number of human parechovirus (HPeV) cases in infants and young children by month, Freiburg, Germany, 2016–2018.

and Erlangen ($n = 10$) in southern Germany, Bonn ($n = 10$) in western Germany, and Charité Berlin ($n = 14$) in northeastern Germany. We detected 134 HPeV strains in respiratory, fecal, CSF, and serum samples. These were typed based on the VP1 genomic region (9). We detected HPeV types 1, 3, 4, 5, and 6. We deposited all sequences in GenBank under accession numbers MK204942–MK204985 and MK291273–MK291362 (Appendix Tables 1, 2). For HPeV-3 phylogenetic analysis, we included 74 strains identified during 2016–2018 and compared them with representative reference strains available from GenBank (Figure 2). Because of high nucleotide variability in the 3' end of the VP1 coding region, we included only complete VP1 sequences.

As recently described, 2 HPeV-3 lineages have been identified (10). Widespread clustering proved co-circulation of the 2016–2018 HPeV strains from Germany (Figure 2). One cluster comprising German strains was most

closely related to HPeV-3 identified in Japan (98.82% nt identity), Australia (98.82%), and the United Kingdom (99.12%).

Among the 21 Freiburg 2018 HPeV-3 strains, 3 groups of completely identical VP1 sequences (10, 3, and 2 sequences) were observed. However, no separate clustering could be detected among these strains because HPeV sequences from other regions in Germany also were assigned to these groups. A direct epidemiologic link could be drawn between 2 cases (cases 12 and 14, with completely identical VP1 sequences, were in twins; Figure 2). Another 2 cases (cases 15 and 18) shared time on the same ward and also displayed 100% identical sequences. However, no hospital ward-specific clustering was observed, suggesting community-acquired transmission.

Conclusions

Routine diagnostics showed an unexpectedly high number of HPeV cases during a 6-week period in 2 neighboring hospitals in Freiburg, Germany. This raised concern about the possibility of a nosocomial outbreak. Recently, healthcare-associated transmission of HPeV-3 has been described. This makes timely identification of outbreaks essential from a hospital hygiene, as well as a public health, perspective (7).

Several patients showed signs of sepsis-like illness, including the clinical triad of fever, poor feeding, and irritability. This is similar to a UK case series reporting a cluster of HPeV infections among infants in 2016 (6). In our study, HPeV-3 was detected exclusively in CSF samples, indicating a more severe clinical phenotype compared with HPeV-1 and -5 infections, supporting previous data (11). Studies have shown that rapid detection of HPeV reduced length of hospital stay and antimicrobial drug use. This emphasizes the usefulness of HPeV diagnostics (1). We showed that HPeV diagnostics, including molecular typing, helped to exclude a nosocomial outbreak. Diagnostically, plasma, respiratory swab, and fecal samples all showed high detection rates, and most patients were positive in ≥ 1 area. Testing of blood samples for enterovirus detection was recently proposed for infants and should be considered for HPeV accordingly (12).

We demonstrated different HPeV types and sublineages, including 2 rare HPeV-5 infections. By conducting phylogenetic analysis in combination with reviewing epidemiologic data, we could exclude a nosocomial outbreak. However, based on this information, transmissions could not be ruled out in 2 independent events with 2 cases each. Although a cluster of HPeV-3 infections has been described (6), retrospective sequence analysis showed different clustering of the identified strains (13). Because of low nucleotide variability, sequence-based differentiation between HPeV-3 strains remains

Table 1. Epidemiologic data for HPeV cases in Freiburg, Germany, January–September 2018*

Case no.	Patient age, mo/sex	Specimen type				HPeV type
		Cerebrospinal fluid	Upper respiratory tract	Plasma	Feces	
1	2/M	Negative	Positive	NA	Negative	3
2	3/M	Positive	Positive	Positive	Positive	3
3	0/F	Positive	Positive	NA	NA	3
4	19/M	NA	NA	NA	Positive	1
5	1/M	Positive	Positive	Positive	Positive	3
6	1/M	NA	NA	Positive	Positive	3
7	1/F	NA	NA	NA	Positive	3
8	7/M	Negative	NA	NA	Positive	3
9	2/M	Negative	NA	NA	Positive	3
10	2/M	NA	NA	Positive	NA	3
11	1/M	NA	Positive	NA	Positive	3
12	0/M	Positive	Positive	Positive	Positive	3
13	1/M	Positive	Positive	NA	NA	3
14	0/F	Positive	Positive	Positive	Positive	3
15	0/F	NA	NA	Positive	Positive	3
16	2/M	NA	NA	Positive	Positive	3
17	2/M	NA	NA	Positive	Positive	3
18	1/F	NA	NA	Positive	Positive	3
19	17/F	NA	Positive	NA	NA	1
20	1/F	NA	NA	Positive	Positive	3
21	4/F	NA	Positive	NA	Positive	3
22	2/M	Positive	NA	NA	Positive	3
23	0/M	NA	NA	Positive	Positive	5
24	0/M	Positive	NA	NA	Positive	3
25	1/M	NA	NA	Positive	Positive	5

*HPeV, human parechovirus; NA, not applicable (no specimen).

Table 2. Clinical signs and symptoms of HPeV cases in Freiburg, Germany, January–September 2018*

Clinical signs and symptoms	No. (%) positive patients		
	HPeV-1, n = 2	HPeV-3, n = 21	HPeV-5, n = 2
Fever	1 (50)	21 (100)	2 (100)
Poor feeding	1 (50)	16 (76)	1 (50)
Irritability	0	13 (62)	1 (50)
Rash	1 (50)	6 (29)	1 (50)
Diarrhea	1 (50)	5 (24)	0
Respiratory distress	0	5 (24)	0
Vomiting	1 (50)	0	0

*HPeV, human parechovirus

ambiguous, a circumstance that impedes molecular outbreak investigation (14).

Our study has limitations. There is a lack of available sequence data from pediatric patients in Germany. In contrast to reports from the Netherlands and the UK, a biannual cycle of HPeV infections has not been demonstrated in Germany; however, our data suggest a biannual cycle. From a public health perspective, a central repository for HPeV sequences, together with key anonymized clinical data from human cases, would improve our understanding of HPeV epidemiology and virus evolution. Institutionalized surveillance similar to the enterovirus surveillance and typing systems already in place across Europe could serve as a blueprint (8,15).

Our report underscores the usefulness of HPeV diagnostics in infants. It illustrates the power of VP1 sequence-guided phylogenetic HPeV analysis, which helped, in combination with epidemiologic data, to rapidly investigate an HPeV outbreak.

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Dr. Elling is a pediatric infectious diseases specialist at the Centers for Pediatrics and Adolescent Medicine and Chronic Immunodeficiency in Freiburg, Germany. His research interests are pediatric infectious diseases and innate immunity. He is supported by the Berta-Ottenstein Clinician Scientist program of the Medical Faculty, University of Freiburg. Dr. Böttcher is a virologist at the German National Reference Center for Poliomyelitis and Enteroviruses at the Robert Koch-Institute in Berlin, Germany. Her research focuses on molecular virology and epidemiology of enteroviruses and parechoviruses.

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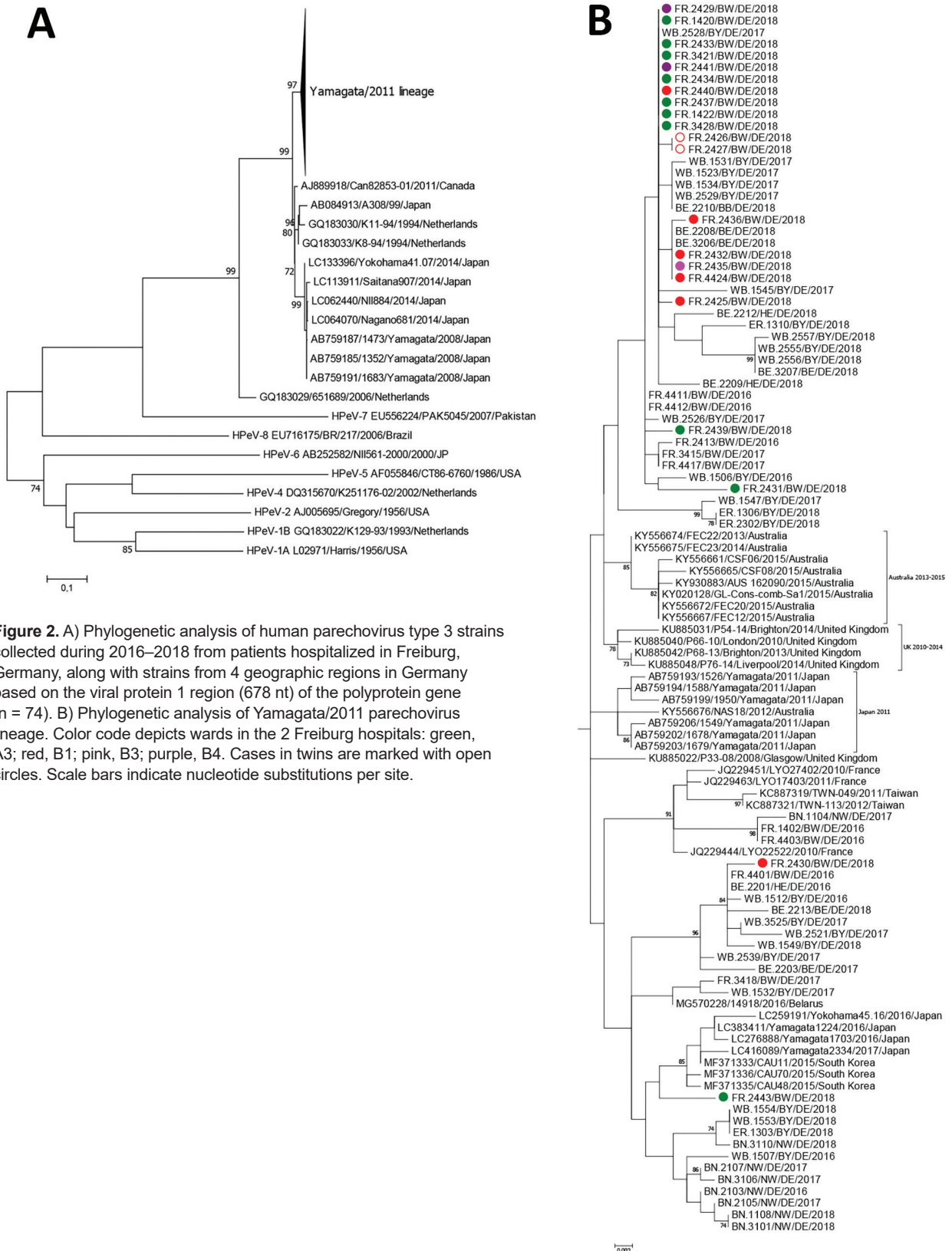


Figure 2. A) Phylogenetic analysis of human parechovirus type 3 strains collected during 2016–2018 from patients hospitalized in Freiburg, Germany, along with strains from 4 geographic regions in Germany based on the viral protein 1 region (678 nt) of the polyprotein gene (n = 74). B) Phylogenetic analysis of Yamagata/2011 parechovirus lineage. Color code depicts wards in the 2 Freiburg hospitals: green, A3; red, B1; pink, B3; purple, B4. Cases in twins are marked with open circles. Scale bars indicate nucleotide substitutions per site.

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EID SPOTLIGHT TOPIC

Antibiotics and similar drugs, together called antimicrobial agents, have been used for the past 70 years to treat patients who have infectious diseases. Since the 1940s, these drugs have greatly reduced illness and death from infectious diseases. However, these drugs have been used so widely and for so long that the infectious organisms the antibiotics are designed to kill have adapted to them, making the drugs less effective.

Each year in the United States, at least 2 million people become infected with bacteria that are resistant to antibiotics and at least 23,000 people die each year as a direct result of these infections.



EMERGING INFECTIOUS DISEASES <http://wwwnc.cdc.gov/eid/page/resistance-spotlight>

Molecular Epidemiology of Human Parechovirus Type 3 Upsurge in 2 Hospitals, Freiburg, Germany, 2018

Appendix

Appendix Table 1. Sample type, GenBank accession number, and strain ID of human parechovirus cases, Freiburg, Germany, January–September 2018*

Case no.	Sample type	Accession no.	Strain ID
1	Upper respiratory tract	MK204975	FR.1420/BW/DE/2018
2	CSF	MK204964	FR.3421/BW/DE/2018
3	Upper respiratory tract	MK204969	FR.1422/BW/DE/2018
4	Fecal	MK204944	FR.2423/BW/DE/2018
5	Fecal	MK204971	FR.2429/BW/DE/2018
6	Fecal	MK204980	FR.2430/BW/DE/2018
7	Fecal	MK204976	FR.2431/BW/DE/2018
8	Fecal	MK204970	FR.2432/BW/DE/2018
9	Fecal	MK204956	FR.2433/BW/DE/2018
10	Plasma	MK204954	FR.4424/BW/DE/2018
11	Fecal	MK204958	FR.2425/BW/DE/2018
12	Fecal	MK204962	FR.2427/BW/DE/2018
13	CSF	MK204963	FR.3428/BW/DE/2018
14	Fecal	MK204957	FR.2426/BW/DE/2018
15	Fecal	MK204966	FR.2434/BW/DE/2018
16	Fecal	MK204955	FR.2435/BW/DE/2018
17	Fecal	MK204972	FR.2436/BW/DE/2018
18	Fecal	MK204968	FR.2437/BW/DE/2018
19	Upper respiratory tract	MK204951	FR.1438/BW/DE/2018
20	Fecal	MK204967	FR.2440/BW/DE/2018
21	Fecal	MK204977	FR.2439/BW/DE/2018
22	Fecal	MK204965	FR.2441/BW/DE/2018
23	Fecal	MK204943	FR.2442/BW/DE/2018
24	Fecal	MK204979	FR.2443/BW/DE/2018
25	Plasma	MK204942	FR.4444/BW/DE/2018

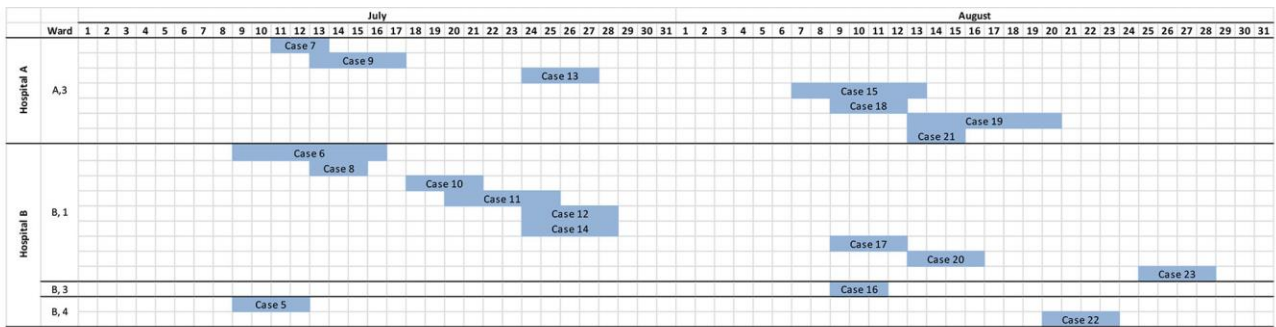
*CSF, cerebrospinal fluid; ID, identification.

Appendix Table 2. GenBank accession number, strain ID, specimen type, year of sampling and human parechovirus (HPEV) type of cases in Bonn (BN), Charité Berlin (BE), Erlangen (ER), Freiburg (FR; 2016–2017 only), and Würzburg (WB), Germany, January 2016–September 2018

Accession no.	Strain ID	Specimen type	Year	HPEV type
MK291287	BN.3101/NW/DE/2018	Cerebrospinal fluid	2018	3
MK291288	BN.1102/NW/DE/2016	Respiratory	2016	1
MK291291	BN.2103/NW/DE/2016	Fecal	2016	3
MK291289	BN.1104/NW/DE/2017	Respiratory	2017	3
MK291292	BN.2105/NW/DE/2017	Fecal	2017	3
MK291295	BN.3106/NW/DE/2017	Cerebrospinal fluid	2017	3
MK291293	BN.2107/NW/DE/2017	Fecal	2017	3
MK291290	BN.1108/NW/DE/2018	Respiratory	2018	3
MK291294	BN.2109/NW/DE/2018	Fecal	2018	1
MK291296	BN.3110/NW/DE/2018	Cerebrospinal fluid	2018	3
MK291275	BE.2201/HE/DE/2016	Fecal	2016	3
MK291273	BE.1202/BE/DE/2016	Respiratory	2016	1
MK291276	BE.2203/BE/DE/2017	Fecal	2017	3
MK291286	BE.4204/BE/DE/2018	Serum	2018	6
MK291274	BE.1205/BE/DE/2018	Respiratory	2018	4
MK291284	BE.3206/BE/DE/2018	Cerebrospinal fluid	2018	3
MK291285	BE.3207/BE/DE/2018	Cerebrospinal fluid	2018	3
MK291277	BE.2208/BE/DE/2018	Fecal	2018	3
MK291278	BE.2209/HE/DE/2018	Fecal	2018	3
MK291279	BE.2210/BB/DE/2018	Fecal	2018	3
MK291280	BE.2211/HE/DE/2018	Fecal	2018	5
MK291281	BE.2212/HE/DE/2018	Fecal	2018	3
MK291282	BE.2213/BE/DE/2018	Fecal	2018	3
MK291283	BE.2214/HE/DE/2018	Fecal	2018	4
MK291297	ER.1301/BY/DE/2018	Respiratory	2018	5
MK291303	ER.2302/BY/DE/2018	Fecal	2018	3
MK291298	ER.1303/BY/DE/2018	Respiratory	2018	3
MK291304	ER.2304/BY/DE/2018	Fecal	2018	3
MK291305	ER.2305/BY/DE/2018	Fecal	2018	5
MK291299	ER.1306/BY/DE/2018	Respiratory	2018	3
MK291300	ER.1307/BY/DE/2018	Respiratory	2018	1
MK291301	ER.1308/BY/DE/2018	Respiratory	2018	1
MK291306	ER.2309/BY/DE/2018	Fecal	2018	5
MK291302	ER.1310/BY/DE/2018	Respiratory	2018	3
MK204981	FR.4401/BW/DE/2016	Plasma	2016	3
MK204985	FR.1402/BW/DE/2016	Respiratory	2016	3
MK204983	FR.4403/BW/DE/2016	Plasma	2016	3
MK204984	FR.2404/BW/DE/2016	Fecal	2016	1
MK204950	FR.1405/BW/DE/2016	Respiratory	2016	1
MK204982	FR.1406/BW/DE/2016	Respiratory	2016	1
MK204949	FR.1407/BW/DE/2016	Respiratory sample	2016	1
MK204948	FR.2408/BW/DE/2016	Fecal	2016	1
MK204953	FR.1409/BW/DE/2016	Respiratory	2016	1
MK204947	FR.2410/BW/DE/2016	Fecal	2016	1
MK204960	FR.4411/BW/DE/2016	Plasma	2016	3
MK204959	FR.4412/BW/DE/2016	Plasma	2016	3
MK204961	FR.2413/BW/DE/2016	Fecal	2016	3
MK204946	FR.1414/BW/DE/2017	Respiratory	2017	1
MK204973	FR.3415/BW/DE/2017	Cerebrospinal fluid	2017	3
MK204952	FR.1416/BW/DE/2017	Respiratory	2017	1
MK204974	FR.4417/BW/DE/2017	Plasma	2017	3
MK204978	FR.3418/BW/DE/2017	Cerebrospinal fluid	2017	3
MK204945	FR.1419/BW/DE/2017	Respiratory	2017	1
MK291307	WB.1501.BY/2016/DEU	Respiratory	2016	1
MK291308	WB.1502.BY/2016/DEU	Respiratory	2016	1
MK291309	WB.1503.BY/2016/DEU	Respiratory	2016	1
MK291310	WB.1504.BY/2016/DEU	Respiratory	2016	1
MK291311	WB.1505.BY/2016/DEU	Respiratory	2016	1
MK291312	WB.1506.BY/2016/DEU	Respiratory	2016	3

Accession no.	Strain ID	Specimen type	Year	HPeV type
MK291313	WB.1507.BY/2016/DEU	Respiratory	2016	3
MK291314	WB.1508.BY/2016/DEU	Respiratory	2016	1
MK291315	WB.1509.BY/2016/DEU	Respiratory	2016	4
MK291316	WB.1510.BY/2016/DEU	Respiratory	2016	1
MK291317	WB.1511.BY/2016/DEU	Respiratory	2016	1
MK291318	WB.1512.BY/2016/DEU	Respiratory	2016	3
MK291319	WB.1513.BY/2016/DEU	Respiratory	2016	1
MK291320	WB.1514.BY/2016/DEU	Respiratory	2016	1
MK291321	WB.1515.BY/2016/DEU	Respiratory	2016	1
MK291322	WB.1516.BY/2017/DEU	Respiratory	2017	1
MK291323	WB.1517.BY/2017/DEU	Respiratory	2017	4
MK291324	WB.1518.BY/2017/DEU	Respiratory	2017	3
MK291325	WB.1519.BY/2017/DEU	Respiratory	2017	1
MK291326	WB.1520.BY/2017/DEU	Respiratory	2017	1
MK291354	WB.2521.BY/2017/DEU	Fecal	2017	3
MK291327	WB.1522.BY/2017/DEU	Respiratory	2017	1
MK291328	WB.1523.BY/2017/DEU	Respiratory	2017	3
MK291329	WB.1524.BY/2017/DEU	Respiratory	2017	1
MK291362	WB.3525.BY/2017/DEU	Cerebrospinal fluid	2017	3
MK291355	WB.2526.BY/2017/DEU	Fecal	2017	3
MK291330	WB.1527.BY/2017/DEU	Respiratory	2017	1
MK291356	WB.2528.BY/2017/DEU	Fecal	2017	3
MK291357	WB.2529.BY/2017/DEU	Fecal	2017	3
MK291331	WB.1530.BY/2017/DEU	Respiratory	2017	1
MK291332	WB.1531.BY/2017/DEU	Respiratory	2017	3
MK291333	WB.1532.BY/2017/DEU	Respiratory	2017	3
MK291334	WB.1533.BY/2017/DEU	Respiratory	2017	1
MK291335	WB.1534.BY/2017/DEU	Respiratory	2017	3
MK291336	WB.1535.BY/2017/DEU	Respiratory	2017	1
MK291337	WB.1536.BY/2017/DEU	Respiratory	2017	6
MK291338	WB.1537.BY/2017/DEU	Respiratory	2017	1
MK291339	WB.1538.BY/2017/DEU	Respiratory	2017	6
MK291358	WB.2539.BY/2017/DEU	Fecal	2017	3
MK291340	WB.1540.BY/2017/DEU	Respiratory	2017	1
MK291341	WB.1541.BY/2017/DEU	Respiratory	2017	6
MK291342	WB.1542.BY/2017/DEU	Respiratory	2017	4
MK291343	WB.1543.BY/2017/DEU	Respiratory	2017	6
MK291344	WB.1544.BY/2017/DEU	Respiratory	2017	6
MK291345	WB.1545.BY/2017/DEU	Respiratory	2017	3
MK291346	WB.1547.BY/2017/DEU	Respiratory	2017	3
MK291347	WB.1548.BY/2018/DEU	Respiratory	2018	1
MK291348	WB.1549.BY/2018/DEU	Respiratory	2018	3
MK291349	WB.1550.BY/2018/DEU	Respiratory	2018	6
MK291350	WB.1551.BY/2018/DEU	Respiratory	2018	6
MK291351	WB.1552.BY/2018/DEU	Respiratory	2018	1
MK291352	WB.1553.BY/2018/DEU	Respiratory	2018	3
MK291353	WB.1554.BY/2018/DEU	Respiratory	2018	3
MK291359	WB.2555.BY/2018/DEU	Fecal	2018	3
MK291360	WB.2556.BY/2018/DEU	Fecal	2018	3
MK291361	WB.2557.BY/2018/DEU	Fecal	2018	3

*ID, identification.



Appendix Figure. Length of stay on assigned wards of 19 patients with human parechovirus, Freiburg, Germany, July 9–August 25, 2018.