Infection, Genetics and Evolution 68 (2019) 280–285

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Short communication

Genetic diversity of human parechoviruses in stool samples, Germany

Corinna Pietsch*, Uwe G. Liebert

Institute of Virology, Leipzig University, Leipzig, Germany

ARTICLE INFO

Keywords:
Molecular epidemiology
Viral gastroenteritis
Phylogenetic analysis
Sepsis-like illness
Genetic diversity
Diarrhoea

ABSTRACT

Human parechoviruses (HPeV) are ubiquitous and mainly occur in early infancy. They are known to cause various clinical manifestations including acute gastroenteritis. To gain insight into the diversity of circulating HPeV genotypes, stool samples from patients (n = 539) with clinical signs of infectious gastroenteritis which showed negative results for other common viral and bacterial enteric pathogens were obtained during three years, 2008 to 2010. Real-time RT-PCR showed HPeV RNA in 34 (6.3%) of the samples. The HPeV detection rate was highest (8.8%) in samples derived from infants and young children under the age of two years. Genotyping was based on VP3/VP1 junction nucleic acid sequences and revealed predominant HPeV-1B (n = 16) and HPeV-3 (n = 12) strains. Those prevailed minor HPeV-6 (n = 3) as well as HPeV-2, −4 and −5 (n = 1, each) strains. To ascertain the assigned HPeV-2 genotype of uncommon strain LPZ04-2008, analysis of complete coding sequences was performed. In complete VP1 analysis strain LPZ04-2008 showed 81.2% nucleic acid identity with HPeV-2 reference strain Williamson. In phylogenetic analysis VP1 of strain LPZ04–2008 clustered with a recent HPeV-2 strain from the UK. Regarding clinical manifestations, severe disease occurred HPeV-1B, −3 and − 6 infections. In conclusion, this paper a high genetic diversity of HPeV in stool samples, including rare strains. The investigation adds data on the whole coding sequences of the rare HPeV-2 strain. Genotyping results confirm previously reported association of more severe illness with HPeV-3 and HPeV-1B strains.

1. Short communication

Human parechoviruses (HPeV) occur world-wide and mainly in early infancy. Most HPeV infections are mild or asymptomatic. The spectrum of clinical disease is comparable to that of enteroviruses and includes gastroenteritis, respiratory disease, meningitis, encephalitis, acute flaccid paralysis, myocarditis as well as sepsis-like illness (Olijve et al., 2018). Different specimens including CSF, urine, blood, respiratory material, and stool can be used in HPeV diagnostics (de Crom et al., 2016; Harvala et al., 2011). In stool, the duration of virus shedding may reach up to several months (Kapusinszky et al., 2012).

The RNA genome of HPeV is about 7.35 kb in length and subdivided into three regions (P1–3). It contains a single open reading frame (ORF) that encodes a polyprotein which is post-translationally cleaved to yield three structural viral proteins (VP0, VP3, and VP1) and seven non-structural proteins (2A-C and 3A-D) (Ghazi et al., 1998). Analysis of complete genomes identified recombination hotspots between 5′ non-translated region (NTR) and P1, and at P1/P2 junction (Benschop et al., 2010). Different genotypes are distinguished by nucleic acid sequence analysis of the VP1 gene or the VP3/VP1 junction region (Fischer et al., 2014; Nix et al., 2010; Olijve et al., 2018). So far 19 HPeV genotypes have been described (Olijve et al., 2018).

To gain insight into HPeV genotype diversity in in-patients with diarrhoea at Leipzig University Hospital, stool samples were obtained between January 2008 and December 2010. The investigation aimed at HPeV mono-infections associated with gastroenteritis during infancy, childhood and adolescence. Therefore only samples were included that had the following characteristics: (1) derived from patients aged less than 18 years, (2) clinical symptoms of infectious gastroenteritis (diarrhoea, vomiting, abdominal pain), and (3) negativity for nine viruses (genogroup I or II norovirus, rotavirus A, enterovirus, coxsackievirus, echovirus, adenovirus, genogroup I, II, IV, and V sapovirus, and astrovirus), and common enteropathogenic bacteria (*Shigella* spp., *Salmonella* spp., *Yersinia enterocolitica*, and *Campylobacter jejuni*). In total 159, 214 and 166 samples from 2008, 2009 and 2010, respectively, collected from 539 patients met the inclusion criteria. Of these 292 were boys and 247 were girls. A total of 296 (54.9%) patients were below two years of age.

Nucleic acids were extracted from 140 µl of 10% stool suspensions prepared in PBS and eluted in 60 µl elution buffer (NucliSens easyMAG system, bioMérieux, Marcy L'Etoile, France). Human parechovirus 5'NTR RNA was amplified and detected by real-time RT-PCR

* Corresponding author at: Institute of Virology, Leipzig University, Johannisallee 30, 04103 Leipzig, Germany.
E-mail address: corinna.pietsch@medizin.uni-leipzig.de (C. Pietsch).

https://doi.org/10.1016/j.meegid.2019.01.007
Received 29 October 2018; Received in revised form 5 January 2019; Accepted 8 January 2019
Available online 09 January 2019
1567-1348/

Table 1
Human parechovirus (HPeV) RNA detections in stool samples.

Strain	Gender[a]	Age	Sampling month	Clinical symptoms and findings	CT[b]	Type	GenBank accession
LPZ01–2008	m	12 months	Jan-08	Diarrhoea, vomiting, fever	24.3	1B	HM996975
LPZ02-2008	f	15 months	Jan-08	Diarrhoea, fever, febrile seizures, respiratory disease	40.0	1B	HM996976
LPZ03-2008	m	7 months	Jun-08	Diarrhoea, vomiting, fever	21.6	1B	HM996977
LPZ04-2008	m	2 years	Jun-08	Diarrhoea, vomiting	25.9	2	HM996978
LPZ05-2008	f	6 years	Jun-08	Abdominal pain, fever	32.6	4	HM996979
LPZ06-2008	m	1 month	Jul-08	Diarrhoea, fever, rash, sepsis-like illness	26.8	3	HM996980
LPZ07-2008	f	17 months	Aug-08	Flatulence, abdominal pain, fever	31.0	3	HM996981
LPZ08-2008	m	3 months	Oct-08	Vomiting, obstipation	23.8	1B	HM996982
LPZ09-2008	m	13 months	Oct-08	Diarrhoea, fever, febrile seizures, respiratory disease	32.3	6	HM996983
LPZ10-2008	f	2 years	Nov-08	Diarrhoea, fever, respiratory disease, rash	33.7	1B	HM996984
LPZ11-2008	f	2 months	Dec-08	Diarrhoea, fever, respiratory disease	33.0	3	HM996985
LPZ12-2009	m	2 years	Jan-09	Diarrhoea, vomiting	38.0	6	HM996986
LPZ13-2009	m	22 months	Feb-09	Diarrhoea, vomiting, fever	17.6	1B	HM996987
LPZ14-2009	m	1 month	Feb-09	Diarrhoea, vomiting	30.0	1B	HM996988
LPZ15-2009	f	14 months	Jul-09	Diarrhoea, vomiting, respiratory disease	28.0	1B	JQ927554
LPZ16-2009	m	8 days	Sep-09	Diarrhoea, rash, sepsis-like illness	27.2	3	HM996989
LPZ17-2009	m	1 month	Sep-09	Vomiting	15.1	1B	HM996990
LPZ18-2009	m	4 years	Oct-09	Diarrhoea, vomiting, fever, intussusception	31.1	6	HM996991
LPZ19-2009	f	7 months	Nov-09	Diarrhoea, vomiting, fever, respiratory disease	25.3	1B	HM996992
LPZ20-2009	m	18 months	Dec-09	Diarrhoea, vomiting, fever, intussusception	32.6	1B	JQ927555
LPZ21-2010	m	6 months	Feb-10	Diarrhoea, vomiting	18.7	1B	JQ927556
LPZ22-2010	m	7 months	Feb-10	Diarrhoea, fever, respiratory disease	30.0	1B	JQ927557
LPZ23-2010	m	14 days	May-10	Diarrhoea, fever, respiratory disease	30.2	3	JQ927558
LPZ24-2010	f	14 months	Jul-10	Diarrhoea, vomiting, fever, respiratory disease	28.0	5	JQ927559
LPZ25-2010	m	28 days	Jul-10	Diarrhoea, vomiting, fever, rash	30.0	3	JQ927560
LPZ26-2010	m	2 months	Aug-10	Diarrhoea, fever, respiratory disease	28.5	3	JQ927561
LPZ27-2010	f	1 month	Aug-10	Diarrhoea, fever, sepsis-like illness	28.0	3	JQ927562
LPZ28-2010	f	6 years	Aug-10	Diarrhoea, vomiting, fever, respiratory disease	27.0	3	JQ927563
LPZ29-2010	f	5 months	Aug-10	Diarrhoea	24.3	1B	JQ927564
LPZ30-2010	m	22 months	Sep-10	Vomiting	19.2	1B	JQ927565
LPZ31-2010	m	5 years	Oct-10	Abdominal pain, afebrile seizures	23.4	3	JQ927566
LPZ32-2010	f	2 years	Oct-10	Diarrhoea	30.0	3	JQ927567
LPZ33-2010	m	5 months	Oct-10	Diarrhoea, fever, respiratory disease	16.6	3	JQ927568
LPZ34-2010	m	1 month	Dec-10	Diarrhoea	20.2	1B	JQ927569

[a] f, female; m, male.
[b] CT, cycle threshold value of real-time RT-PCR.

(QuantiFast Probe RT-PCR + ROXVialKit, Qiagen, Hilden, Germany) following the manufacturer's instructions on a LightCyler 2.0 instrument (Roche Diagnostics GmbH, Mannheim, Germany) using consensus primers and adjacent hybridization probes (Table S1). Real-time RT-PCR showed positive results in 34 (6.3%) stools, i.e. 6.9%, 4.2%, and 8.4% of the samples collected in 2008, 2009, and 2010, respectively. Human parechoviruses were detected from May to February and tended to be more frequent during summer and autumn months (Table 1). There was no significant difference in positivity rate in samples derived from boys (7.5%) and from girls (4.9%). The age of the HPeV positive patients ranged from 8 days to 6 years, with highest detection rates in infants under the age of 2 years (8.8%), in line with previous reports (Baumgarte et al., 2008; Fischer et al., 2014).

For genotyping, the VP3/VP1 junction of HPeV genome was amplified using consensus primers (Table S1). Amplicons were gel purified (Wizard SV Gel and PCR Clean-Up System, Promega, Mannheim, Germany) and sequenced (BigDye Terminator v1.1 Cycle Sequencing kit and ABI Prism 310 Genetic Analyzer, Applied Biosystems, Foster City, CA). In samples with low viral load, the gel-purified amplicon was re-amplified prior to sequencing. Sequences were submitted to GenBank (Table 1), aligned to other GenBank accessions and phylogenetically analyzed using MEGA5 (Tamura et al., 2011). This approach was successful in all HPeV positive samples. Consistent with recent data from Europe, HPeV-1B and HPeV-3 genotypes predominated (Harvala et al., 2011; van der Sanden et al., 2013). As reported previously, HPeV-3 strains mainly circulated in even-numbered years (Harvala et al., 2008), pointing to a possible biannual seasonality of the genotype in the study area. In total, phylogenetic analysis identified 16 (47.1%) type 1B, twelve (35.3%) type 3, and three (8.8%) type 6 HPeV. Genotype HPeV-2, HPeV-4 and HPeV-5 strains were detected once each (2.9%) (Fig. 1). Different variants co-circulated within the HPeV-1B, HPeV-3 and HPeV-6 genotypes (Fig. 1). The present genetic diversity of HPeV in stool samples was higher than previously reported for Germany (Baumgarte et al., 2008), but consistent with more recent data from Italy and the Netherlands (Piralla et al., 2012; van der Sanden et al., 2013). In contrast to stools collected during similar sampling periods in Asia, no HPeV-1A strains were detected (Guo et al., 2013; Alam et al., 2015; Patil et al., 2018).

As VP3/VP1 nucleic acid identity of strain LPZ04-2008 and HPeV reference strain Williamson (78.9%) was close to the nucleic acid divergence threshold applied in HPeV genotyping, sequences of overlapping PCR amplicons spanning the complete coding genome of the virus and partial 3'/5'NTR were obtained (Table S1). Comparison to HPeV reference strains confirmed the HPeV-2 genotype showing highest nucleotide identities with strain Williamson across all P1 genes. None of the analyzed HPeV reference strains showed high nucleic acid identities to LPZ04-2008 in all non-structural polyprotein genes (Table 2), possibly due to past recombination events (Benschop et al., 2010). In phylogenetic analysis of all available complete HPeV-2 VP1 sequences, strain LPZ04-2008 clustered with strain P5–14, detected 2014 in the UK (Fig. 2). An extended literature review revealed HPeV-2 genotype reports in 46 stool samples and few available HPeV-2

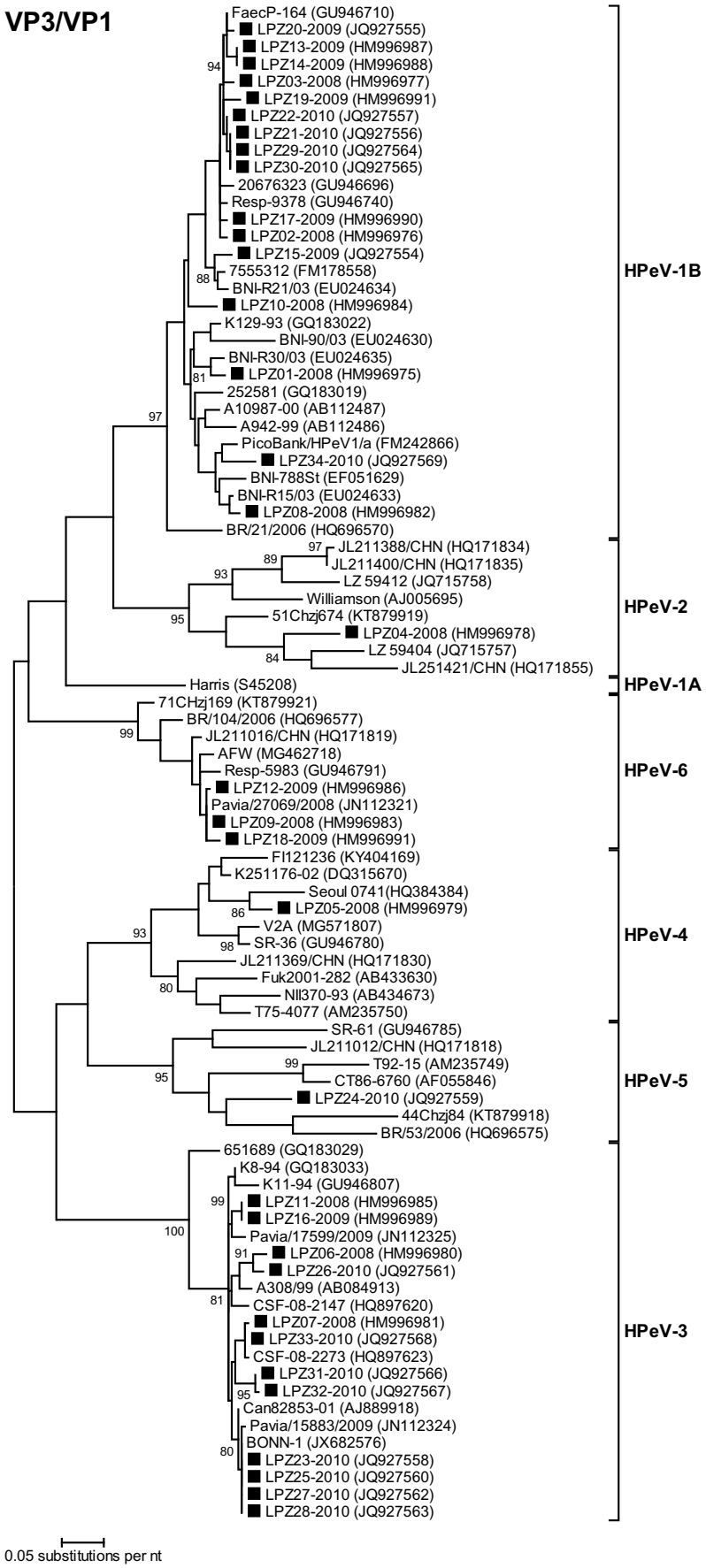

Fig. 1. Phylogenetic analysis of VP3/VP1 junction at nucleotide level (256–259 nucleotides). The filled squares indicate the present HPeV strains. The dendrogramme was constructed by the Maximum-Likelihood method based on Tamura-Nei model. Statistical support was assessed by bootstrapping with 1000 replicates. The bootstrap values over 80 are shown. GenBank accessions are given in brackets.

Table 2
Similarity of human parechovirus (HPeV) reference strains to the genome of strain LPZ04-2008.

Genotype	Strain	VP0	VP3	VP1	2A	2B	2C	3A	3B	3C	3D
HPeV-1	Harris	73.7	75.4	68.5	74.5	81.2	75.4	76.1	72.1	82.2	82.7
HPeV-1	BNI-788St	74.1	73.4	68.7	**78.9**	82.3	76.5	74.4	85.2	85.4	88.4
HPeV-2	Williamson	**81.1**	**80.2**	**81.2**	76.1	75.7	**78.6**	74.1	73.8	80.5	83.0
HPeV-3	A308/99	70.2	69.7	66.0	74.9	81.5	75.8	71.0	78.7	86.2	89.5
HPeV-4	K251176–02	73.4	74.0	68.4	77.2	**83.1**	77.1	76.4	78.7	86.9	**90.4**
HPeV-5	CT86–6760	72.6	69.2	66.3	75.4	79.8	76.2	70.7	78.7	80.7	82.1
HPeV-6	BNI67/03	73.1	70.2	65.2	73.8	80.6	76.4	73.5	75.0	81.2	82.6
HPeV-7	PAK5045	69.9	68.9	66.7	73.8	79.8	77.4	77.0	**90.2**	**87.2**	89.8
HPeV-8	BR/217/2006	72.5	69.1	67.7	78.3	79.0	75.8	73.6	75.4	81.7	84.2
HPeV-14	V3C	72.3	67.7	65.7	75.1	79.2	76.5	75.2	78.3	84.5	83.2
HPeV-17	M36/CI/2014	71.6	71.5	68.9	71.8	76.5	76.1	78.1	76.7	78.9	83.4
HPeV-18	11Chzj207	70.7	71.0	68.6	75.6	79.8	77.3	**79.4**	81.0	86.4	87.8
HPeV-19	67Chzj11	71.4	69.5	68.0	75.5	79.2	76.7	74.6	84.1	84.4	82.4

The highest percentage within each column is indicated by bold type.

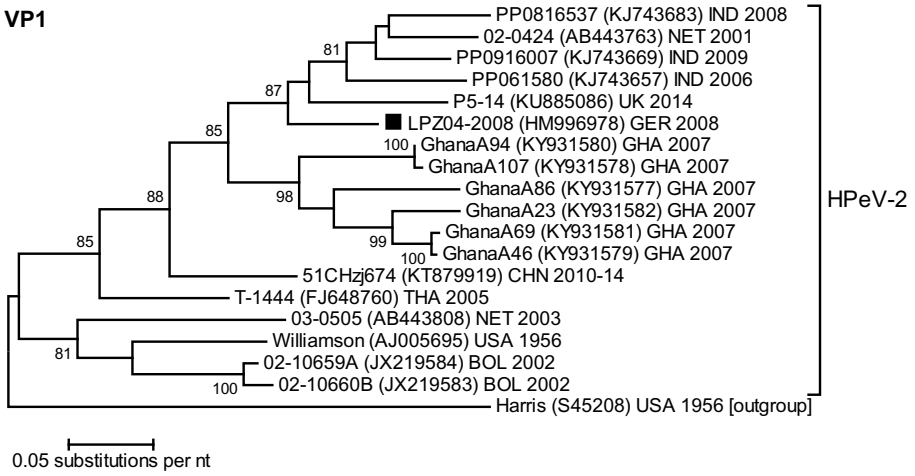

Fig. 2. Phylogenetic analysis of the complete VP1 gene of human parechovirus-2 (HPeV-2) strains at nucleotide level (689 nucleotides). The filled square indicates the present HPeV-2 strain LPZ04–2008. The dendrogramme was constructed by the Maximum-Likelihood method based on Tamura-Nei model. Statistical support was assessed by bootstrapping with 1000 replicates. The bootstrap values over 80 are shown. GenBank accessions are given in brackets. The country of sample origin (BOL, Bolivia; CHN, China; GER, Germany; GHA, Ghana; IND, India; NET, Netherlands; THA, Thailand; UK, United Kingdom; USA, United States of America) and year of sample collection are added.

sequences, including two complete genomes (Table 3). In additional 245 samples, mainly of respiratory origin, HPeV-2 was reported based on serotyping results. Yet, HPeV genotyping and serotyping does not fully correspond (Westerhuis et al., 2015). Thus, genotyping had disproved several early serotype-based reports of HPeV-2 in respiratory samples and confirmed HPeV-2 exclusively in fecal samples (Table 3) (Abed and Boivin, 2006; Oberste et al., 1998). We therefore assume, that HPeV-2 genotypes are not related to respiratory illness. In agreement with recently reviewed data (Olijve et al., 2018), clinical manifestation was mild in the present HPeV-2 positive patient. The boy vomited and was admitted to hospital to exclude contusion, as a minor head injury coincided.

Infectious gastroenteritis was accompanied with respiratory disease in infections caused by HPeV types 1B, 3, 5 and 6 (n = 12, 35.3%) in the present study. Severe diseases (sepsis-like illness, seizures, intussusception) occurred in HPeV-1B, −3 and −6, but not in HPeV-2, −4, and −5 infections (Table 1). An association of severe disease with HPeV-3 and HPeV-1B strains has been described previously (Harvala et al., 2009; Jeziorsky et al., 2014; Momoki, 2018). And in line with previous reports, all present cases of sepsis-like illness were associated with HPeV-3 (Abed and Boivin, 2006; Harvala et al., 2011). The present data, as well as recent results from a study in patients with suspected meningitis (Karsch et al., 2015), additionally point to a possible association of HPeV-6 genotypes with more severe disease.

In conclusion, this paper shows a high genetic diversity of HPeV in stool samples, including rare strains. The investigation adds sequencing data of a rare HPeV-2 strain. Genotyping results confirm previously reported association of more severe illness with HPeV-3 and HPeV-1B strains.

Competing interests

Nothing to declare.

Ethical clearance

The study was approved by the Ethics Committee of Leipzig University (AZ 298/16-ek).

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Table 3
Overview of data about published human parechovirus-2 (HPeV-2) detections.

Year	Country	Specimen (no.)	Strain ID	Supporting data[a]	VP1 nt. identity to LPZ04-2008[c]	Ref.
1956	USA	Faeces (1)	Williamson	Serotyping, complete coding sequence	81.2%	(Ghazi et al., 1998; Wigand and Sabin, 1961)
1975–1994	UK	n/a (169)	n/a	Serotyping[b]	n/a	(Maguire et al., 1999)
1980	Italy	Resp. (4)	9445, 9681, 9917, 9991	Serotyping[b]	n/a	(Barbi Guidotti et al., 1982)
1961–1994	Sweden	Faeces (4), resp. (1)	n/a	Serotyping[b]	n/a	(Ehrnst and Eriksson, 1996)
1970–2005	USA	Resp. and n/a (60)	n/a	Serotyping[b]	n/a	(Khetsuriani et al., 2006)
1980–1994	Belgium	n/a (5)	n/a	Serotyping[b]	n/a	(Druyts-Voets, 1997)
1998–2009	Netherlands	Faeces (n/a)	n/a	Serotyping[b]	n/a	(van der Sanden et al., 2013)
2001	Canada	Faeces (1)	Can82047–01	Serotyping, VP3	n/a	(Abed and Boivin, 2006)
2002/2003	Netherlands	Faeces (2)	02–0424, 03–0505	VP1 + 2A(p)	88.6%, 80.9%	(van der Sanden et al., 2008)
2002/2003	Bolivia	Faeces (8)	02-10659A, 02-10660B	VP1	81.6%, 81.3%	(Nix et al., 2013)
2005	Thailand	Faeces (1)	T-144	VP1 + VP2A(p)	83.3%	(Pham et al., 2010)
2006	Norway	Faeces (1)	NO-13631	VP1(p)	(88.1%)	(Tapia et al., 2008)
2006–2009	India	Faeces (4)	PP061580, PP0916007, PP0816537; PP0713580	VP3(p) + VP1 + VP2A(p); VP1(p)	87.1%, 89.6%, 87.2%; (89.3%)	(Patil et al., 2018)
2007	Ghana	Faeces (6)	GhanaA23, −46, −69, −86, −94, −107	VP3(p) + VP1 + VP2A(p)	83.8%, 85.7%, 85.2%, 84.8%, 84.2%, 83.8%	(Graul et al., 2017)
2007/2008	Bangladesh	Faeces (5)	14636B, 14654B, 14,738, 14765B, 14766B	VP1(p)	(85.2%, 84.4%, 84.8%, 85.7%, 84.4%)	(Oberste et al., 2013)
2008	Germany	Faeces (1)	LPZ04–2008	Complete coding sequence	–	This study
2008	China	Faeces (3)	JL211388, JL211400, JL251421	VP3(p) + VP1(p)	(81.3%, 82.1%, 77.8%)	GenBank HQ171834, −35, −55
2009	China	Faeces (2)	LZ-59404, LZ-59412	VP3(p) + VP1(p)	(87.0%, 80.5%)	(Guo et al., 2013)
2009/2010	Pakistan	Faeces (1)	NIHPAK-RGH2650	VP1[d]	n/a[d]	(Alam et al., 2015)
2009/2011	Netherlands	Faeces (2)	950912_ADAM_NL09, 1151782_ADAM_NL11	VP3(p) + VP1(p)	(85.6%, 89.7%)	(Janes et al., 2014)
2010	Thailand	Faeces (5)	CU-B847, CU-B850, CU-B852, CU-B865, CU-B867	VP3(p) + VP1(p), 3D(p)	(78.5%)	(Chieochansin et al., 2011)
2011–2014	China	Faeces (1)	51CHzj674	Complete coding sequence	85.7%	(Zhao et al., 2016)
2014	India	Faeces (2)	K-291, J-200	VP1(p)	(88.6%)	(Itta et al., 2017)
2014	UK	Faeces (1)	P5–14	VP1	87.2%	GenBank KU885086
2014	Japan	Faeces (1)	Po14–27	VP1(p)	(84.0%)	(Yamamoto et al., 2015)

[a] In case of genotyping the gene regions sequenced in the study are indicated. n/a, not available; (p), partial; resp., respiratory.
[b] No sequence data available.
[c] Identity results, which are based on partial VP1 nucleic acid sequences, are shown in brackets.
[d] Identification of HPeV2 was done by genotyping but nucleic acid sequences are not available in GenBank.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2019.01.007.

References

Abed, Y., Boivin, G., 2006. Human parechovirus types 1, 2 and 3 infections in Canada. Emerg. Infect. Dis. 12, 969–975.

Alam, M.M., Khurshid, A., Shaukat, S., Rana, M.S., Sharif, S., Angez, M., Nisar, N., Aamir, U.B., Naeem, M., Zaidi, S.S., 2015. Viral etiologies of acute dehydrating gastroenteritis in pakistani children: confounding role of parechoviruses. Viruses 7, 378–393.

Barbi Guidotti, M., Cappellini, D., Pedretti, C.C., Ferrante, P., 1982. Spread of echovirus 23 in a neonatal pathology unit. Ann. Sclavo 24, 76–84.

Baumgarte, S., de Souza Luna, L.K., Grywna, K., Panning, M., Drexler, J.F., Karsten, C., Huppertz, H.I., Drosten, C., 2008. Prevalence, types, and RNA concentrations of human parechoviruses, including a sixth parechovirus type, in stool samples from patients with acute enteritis. J. Clin. Microbiol. 46, 242–248.

Benschop, K.S., de Vries, M., Minnaar, R.P., Stanway, G., van der Hoek, L., Wolthers, K.C., Simmonds, P., 2010. Comprehensive full-length sequence analyses of human parechoviruses: diversity and recombination. J. Gen. Virol. 91, 145–154.

Chieochansin, T., Vichiwattana, P., Korkong, S., Theamboonlers, A., Poovorawan, Y., 2011. Molecular epidemiology, genome characterization, and recombination event of human parechovirus. Virology 421, 159–166.

de Crom, S.C., Rossen, J.W., de Moor, R.A., Veldkamp, E.J., van Furth, A.M., Obihara, C.C., 2016. Prospective assessment of clinical symptoms associated with enterovirus and parechovirus genotypes in a multicenter study in Dutch children. J. Clin. Virol. 77, 15–20.

van der Sanden, S., de Bruin, E., Vennema, H., Swanink, C., Koopmans, M., van der Avoort, H., 2008. Prevalence of human parechovirus in the Netherlands in 2000 to 2007. J. Clin. Microbiol. 46, 2884–2889.

van der Sanden, S.M., Koopmans, M.P., van der Avoort, H.G., 2013. Detection of human enteroviruses and parechoviruses as part of the national enterovirus surveillance in the Netherlands, 1996-2011. Eur. J. Clin. Microbiol. Infect. Dis. 32, 1525–1531.

Druyts-Voets, E., 1997. Epidemiological features of entero non-poliovirus isolations in Belgium 1980-94. Epidemiol. Infect. 119, 71–77.

Ehrnst, A., Eriksson, M., 1996. Echovirus type 23 observed as a nosocomial infection in infants. Scand. J. Infect. Dis. 28, 205–206.

Fischer, T.K., Midgley, S., Dalgaard, C., Nielsen, A.Y., 2014. Human parechovirus infection, Denmark. Emerg. Infect. Dis. 20, 83–87.

Ghazi, F., Hughes, P.J., Hyypia, T., Stanway, G., 1998. Molecular analysis of human parechovirus type 2 (formerly echovirus 23). J. Gen. Virol. 79, 2641–2650 Pt 11.

Graul, S., Bottcher, S., Eibach, D., Krumkamp, R., Kasmaier, J., Adu-Sarkodie, Y., May, J., Tannich, E., Panning, M., 2017. High diversity of human parechovirus including novel types in stool samples from Ghanaian children. J. Clin. Virol. 96, 116–119.

Guo, Y., Duan, Z., Qian, Y., 2013. Changes in human parechovirus profiles in hospitalized children with acute gastroenteritis after a three-year interval in Lanzhou, China. PLoS One 8, e68321.

Harvala, H., Robertson, I., McWilliam Leitch, E.C., Benschop, K., Wolthers, K.C., Templeton, K., Simmonds, P., 2008. Epidemiology and clinical associations of human parechovirus respiratory infections. J. Clin. Microbiol. 46, 3446–3453.

Harvala, H., Robertson, I., Chieochansin, T., McWilliam Leitch, E.C., Templeton, K., Simmonds, P., 2009. Specific association of human parechovirus type 3 with sepsis and fever in young infants, as identified by direct typing of cerebrospinal fluid samples. J. Infect. Dis. 199, 1753–1760.

Harvala, H., McLeish, N., Kondracka, J., McIntyre, C.L., McWilliam Leitch, E.C., Templeton, K., Simmonds, P., 2011. Comparison of human parechovirus and enterovirus detection frequencies in cerebrospinal fluid samples collected over a 5-year period in Edinburgh: HPeV type 3 identified as the most common picornavirus type. J. Med. Virol. 83, 889–896.

Itta, K.C., Ghargi, K.V., Kalal, S., Patil, T., Roy, S., 2017. First detection of human parechovirus infection with diarrhoea, India. Infect. Dis. (Lond.) 49, 151–152.

Janes, V.A., Minnaar, R., Koen, G., van Eijk, H., Dijkman-de Haan, K., Pajkrt, D., Wolthers, K.C., Benschop, K.S., 2014. Presence of human non-polio enterovirus and parechovirus genotypes in an Amsterdam hospital in 2007 to 2011 compared to national and international published surveillance data: a comprehensive review. Euro Surveill. 19.

Jeziorsky, E., Schuffenecker, I., Bohrer, S., Pain, J.B., Segondy, M., Foulongne, V., 2014. Relevance of human parechovirus detection in cerebrospinal fluid samples from young infants with sepsis-like illness. J. Clin. Lab. Anal. 29 (2), 112–115.

Kapusinszky, B., Minor, P., Delwart, E., 2012. Nearly constant shedding of diverse enteric viruses by two healthy infants. J. Clin. Microbiol. 50, 3427–3434.

Karsch, K., Obermeier, P., Seeber, L., Chen, X., Tief, F., Muhlhans, S., Hoppe, C., Conrad, T., Bottcher, S., Diedrich, S., Rath, B., 2015. Human parechovirus infections associated with seizures and rash in infants and toddlers. Pediatr. Infect Dis 34, 1049–1055.

Khetsuriani, N., Lamonte-Fowlkes, A., Oberst, S., Pallansch, M.A., Centers for Disease, C., Prevention, 2006. Enterovirus surveillance—United States, 1970-2005. MMWR Surveill. Summ. 55, 1–20.

Maguire, H.C., Atkinson, P., Sharland, M., Bendig, J., 1999. Enterovirus infections in England and Wales: laboratory surveillance data: 1975 to 1994. Commun. Dis. Public Health 2, 122–125.

Momoki, T.S., 2018. Analysis of Human Parechovirus Genotypes in Yokohama District from 2000 to 2016. Jpn. J. Infect. Dis. 71, 298–301.

Nix, W.A., Maher, K., Pallansch, M.A., Oberste, M.S., 2010. Parechovirus typing in clinical specimens by nested or semi-nested PCR coupled with sequencing. J. Clin. Virol. 48, 202–207.

Nix, W.A., Khetsuriani, N., Penaranda, S., Maher, K., Venczel, L., Cselko, Z., Freire, M.C., Cisterna, D., Lema, C.L., Rosales, P., Rodriguez, J.R., Rodriguez, W., Halkyer, P., Ronveaux, O., Pallansch, M.A., Oberste, M.S., 2013. Diversity of picornaviruses in rural Bolivia. J. Gen. Virol. 94, 2017–2028.

Oberste, M.S., Maher, K., Pallansch, M.A., 1998. Complete sequence of echovirus 23 and its relationship to echovirus 22 and other human enteroviruses. Virus Res. 56, 217–223.

Oberste, M.S., Feeroz, M.M., Maher, K., Nix, W.A., Engel, G.A., Hasan, K.M., Begum, S., Oh, G., Chowdhury, A.H., Pallansch, M.A., Jones-Engel, L., 2013. Characterizing the picornavirus landscape among synanthropic nonhuman primates in Bangladesh, 2007 to 2008. J. Virol. 87, 558–571.

Olijve, L., Jennings, L., Walls, T., 2018. Human parechovirus: an increasingly recognized cause of sepsis-like illness in young infants. Clin. Microbiol. Rev. 31.

Patil, P.R., Ganorkar, N.N., Gopalkrishna, V., 2018. Epidemiology and genetic diversity of human parechoviruses circulating among children hospitalised with acute gastroenteritis in Pune, Western India: a 5-years study. Epidemiol. Infect. 146, 11–18.

Pham, N.T., Trinh, Q.D., Khamrin, P., Maneekarn, N., Shimizu, H., Okitsu, S., Mizuguchi, M., Ushijima, H., 2010. Diversity of human parechoviruses isolated from stool samples collected from Thai children with acute gastroenteritis. J. Clin. Microbiol. 48, 115–119.

Piralla, A., Furione, M., Rovida, F., Marchi, A., Stronati, M., Gerna, G., Baldanti, F., 2012. Human parechovirus infections in patients admitted to hospital in Northern Italy, 2008-2010. J. Med. Virol. 84, 686–690.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28, 2731–2739.

Tapia, G., Cinek, O., Witso, E., Kulich, M., Rasmussen, T., Grinde, B., Ronningen, K.S., 2008. Longitudinal observation of parechovirus in stool samples from Norwegian infants. J. Med. Virol. 80, 1835–1842.

Westerhuis, B.M., Benschop, K.S., Koen, G., Claassen, Y.B., Wagner, K., Bakker, A.Q., Wolthers, K.C., Beaumont, T., 2015. Human memory B cells producing potent cross-neutralizing antibodies against human parechovirus: implications for prevalence, treatment, and diagnosis. J. Virol. 89, 7457–7464.

Wigand, R., Sabin, A.B., 1961. Properties of ECHO types 22, 23 and 24 viruses. Arch. Gesamte Virusforsch 11, 224–247.

Yamamoto, S.P., Kaida, A., Naito, T., Hosaka, T., Miyazato, Y., Sumimoto, S., Kohdera, U., Ono, A., Kubo, H., Iritani, N., 2015. Human parechovirus infections and child myositis cases associated with genotype 3 in Osaka City, Japan, 2014. J. Med. Microbiol. 64, 1415–1424.

Zhao, X., Shi, Y., Xia, Y., 2016. Genome analysis revealed novel genotypes and recombination of the human parechoviruses prevalent in children in Eastern China. Gut Pathog. 8, 52.