Genomic characterization of coxsackievirus A22 from a regional university hospital in the Netherlands

Cassidy, Hayley; Schuele, Leonard; Niesters, Hubert G. M.; Van Leer-Buter, Coretta; Lizarazo-Forero, Erley

Published in:
Journal of Clinical Virology

DOI:
10.1016/j.jcv.2022.105272

Publication date:
2022

Journal of Clinical Virology 156 (2022) 105272

Contents lists available at ScienceDirect

Journal of Clinical Virology

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

Genomic characterization of coxsackievirus A22 from a regional university hospital in the Netherlands

Hayley Cassidy, Leonard Schuele, Hubert GM Niesters *, Coretta Van Leer-Buter, Erley Lizarazo-Forero

University of Groningen, University Medical Center Groningen, Department of Medical Microbiology and Infection Prevention, The Netherlands

ARTICLE INFO

Keywords:
Enterovirus
Coxsackievirus A22
Next-generation sequencing
Prolonged infection

ABSTRACT

Background: Enteroviruses are highly diverse with a wide spectrum of genotypes and clinical manifestations. Coxsackievirus A22 (CVA22) has been detected globally from sewage surveillance; however, currently there is limited information on its prevalence in patients, as well as available genomic data.
Objective: We aimed to provide genomic and relative frequency data on CVA22 from a regional hospital perspective between 2013–2020.
Study design: Sanger sequencing was performed on all samples with a positive enterovirus RT-qPCR result (≤Ct 32). Viral targeted sequence capture (ViroCap) and next-generation sequencing (NGS) (Illumina NextSeq 500) was used to characterize and generate near-complete CVA22 genomes for enteroviruses without genotyping results from Sanger sequencing. Epidemiological and phylogenetic analysis was performed using maximum likelihood trees on MEGA-11.
Results: A total of twenty detections derived from fecal material from sixteen patients were observed between 2013– 2020. One transplant recipient had five different CVA22 infection episodes over five years, with phylogenetic analysis indicating possible reinfection with an additional prolonged infection (>3 weeks). Furthermore, we report the first two near-complete CVA22 sequences from Europe, which grouped with a strain previously isolated from Bangladesh in 1999.
Conclusions: We show a highly diverse enterovirus genotype which causes infections annually, typically in autumn and winter, and is capable of recurrent infection in an immunocompromised patient. Furthermore, we highlight the use of NGS to complement conventional targeted Sanger sequencing.

Abbreviations

CVA22	Coxsackievirus A22
NGS	Next-generation sequencing
GI	Gastrointestinal
EV-C	Enterovirus group-C
VP1	Viral protein 1
CVA1	Coxsackievirus A1
CVA19	Coxsackievirus A19
Ct	Cycle threshold

1. Introduction

Enteroviruses are highly diverse with over 120 genotypes globally [1]. Most patients present with asymptomatic, mild indiscriminate gastrointestinal (GI) or respiratory symptoms; however, some infections can lead to severe morbidity and mortality [2]. Enteroviruses that infect humans are categorized into enterovirus groups A-D and typically characterized by targeting the viral protein 1 (VP1) gene on the viral capsid during Sanger sequencing [3]. A total of twenty-three genotypes are classified into enterovirus group-C (EV-C) [1]. Human coxsackievirus A22 (CVA22) is a group-C enterovirus consisting of 7,4 kb-long single-stranded RNA. EV-C can be divided into three distinct groups, depending on phylogenetics and replication properties [4]. CVA22, together with CVA1 and CVA19, is classified within EV-C group II. Limited data currently exists on CVA22 prevalence and spectrum of disease, possibly due to the fact that EV-C group II is difficult to grow in laboratories and surveillance has been historically depended on cell

* Corresponding author at: University of Groningen, University Medical Center Groningen, Department of Medical Microbiology and Infection Prevention, Hanzeplein 1, Groningen, 9713 GZ, The Netherlands.
E-mail address: h.g.m.niesters@umcg.nl (H.G. Niesters).

https://doi.org/10.1016/j.jcv.2022.105272
Received 31 March 2022; Received in revised form 19 August 2022;
Available online 30 August 2022

Table 1
Overview of CVA22 detections in the UMCG between 2013–2020.

Sample ID[*1]	Sex	Age[*2]	Sample material	Ct value[*3]	Sequencing approach	Sample collection	Total no. of typed EV's/ year (VP1)[*4]	Relative frequency at UMCG
NL 1	Male	37	Fecal	32	Sanger	06/2013	92	1.09%
NL 2	Male	76	Fecal	21	Sanger	07/2014	276	1.09%
NL 3	Female	66	Fecal	23	Sanger	10/2014		
NL 4	Female	50	Fecal	14	Sanger	12/2014		
NL 5	Female	3	Fecal	17	Sanger	01/2015	168	2.98%
NL 6	Male	30	Fecal	13	Sanger	04/2015		
NL 7	Female	1	Fecal	23	Sanger	10/2015		
NL 8	Female	4	Fecal	26	Sanger	10/2015		
NL 9 (ep. 1)*	Female	53	Fecal	13	Sanger	12/2015		
NL 10	Male	3	Fecal	31	Sanger	01/2016	250	0.80%
NL 11	Male	45	Fecal	21	NGS	10/2016		
NL 12	Female	62	Fecal	14	Sanger	04/2017	263	0.38%
NL 13 (ep. 2)*	Female	55	Fecal	18	Sanger	05/2018	254	1.97%
NL 14	Male	74	Fecal	30	NGS	07/2018		
NL 15	Male	84	Fecal	NA	Sanger	10/2018		
NL 16	Male	67	Fecal	27	Sanger	10/2018		
NL 17 (ep. 3)*	Female	56	Fecal	12	NGS	10/2018		
NL 18	Male	70	Fecal	21	Sanger	07/2019	209	0.49%
NL 19 (ep. 4)	Female	57	Fecal	15	Sanger	02/2020	72	2.80%
NL 20 (ep. 5)	Female	58	Fecal	16	Sanger	10/2020		

[*1] NL 4 and NL 8 were below 250 bp and were not represented in the tree.
[*2] Age at the time of sample collection.
[*3] Ct value from laboratory developed GI screen.
[*4] Only enteroviruses with successful genotyping results through VP1 Sanger sequencing were used to create the relative frequency.
[*] Clinical case/data described before [5]. The infection episodes (ep.) depicted from the patient with the prolonged CVA22 infection. **Abbreviation:** EV; enterovirus, VP1; viral protein 1 gene, NA; not available, NL; Netherlands, Ct; Cycle threshold.

culture [1,4].

We present the first near-complete European CVA22 genomes using next-generation sequencing (NGS). Furthermore, we highlight the potential of a prolonged CVA22 infection with reported GI presentation and provide phylogenetic analysis of twenty CVA22 sequences recovered from patients admitted to a regional university hospital in the Netherlands between 2013–2020.

2. Methods

2.1. Patients inclusion and laboratory developed GI screen

Patients with GI symptom(s) are routinely screened for suspected viral GI infections at the University Medical Center Groningen (UMCG). Subsequent laboratory developed GI screens are performed and provide cycle threshold (Ct) values for enterovirus, adenovirus, norovirus, astrovirus, parechovirus and sapovirus (Supplementary materials 1.1 and Table S1). A retrospective study was conducted on detected CVA22 sequences between January 2013 and December 2020 using BioNumerics v6.1 and the patient database system. An infection episode was defined as a single clinical period (≤3 weeks), including symptoms and viral detection (Supplementary materials 1.2) [5].

2.2. Sequencing

Sanger sequencing was performed on all samples with a positive enterovirus detection (≤Ct 32) [3]. Viral targeted sequence capture (ViroCap) [6] and NGS (Illumina NextSeq500, 2 × 76 bp) was performed on untypeable enteroviruses [7].

2.3. Data analysis

CLC Genomics Server v21.0.3 was used to perform trimming, alignment, calculate coverage depth, primer annealing analysis and annotation (Table S2). Trimmed reads were assembled using Genome Detective v1.136 [8] and typed using the Enterovirus Genotyping Tool v1 (https://www.rivm.nl/mpf/typingtool/enterovirus/). Maximum likelihood trees with 1000 bootstraps were constructed using a Kimura-2

Table 2
Overview of NGS CVA22 sequences obtained from untypeable samples using Illumina sequencing (NextSeq 500).

ID	Length (bp)	Genotype (VP1) assignment	Genome coverage	Avg. sequence depth	Best hit on GenBank	Identity to best hit
NL 11	7264	CVA22	98.1%[*2]	3,579x	CVA22	90.10%
NL 14	5395	CVA22	72.9%[*3]	12x	CVA1	92.34%
NL 17[*1]	7376	CVA22	99.6%[*2]	153,706x	CVA22	89.54%

Typing (VP1) was performed using the enterovirus typing tool from the RIVM and Genome Detective. NCBI accession numbers were generated for the near-complete genomes, NL 11 (OM963010) and NL 17 (OM963011). Annotation of the CDS and partial 5′ and 3′ regions was performed using CLC Genomics workbench. **Abbreviations:** CVA22; coxsackievirus A22, Bp; basepairs.
[*1] infection episode 3 in the patient with prolonged infection.
[*2] Genome coverage based on best hit: DQ995647.1.
[*3] Genome coverage based on best hit: JX174177.1.

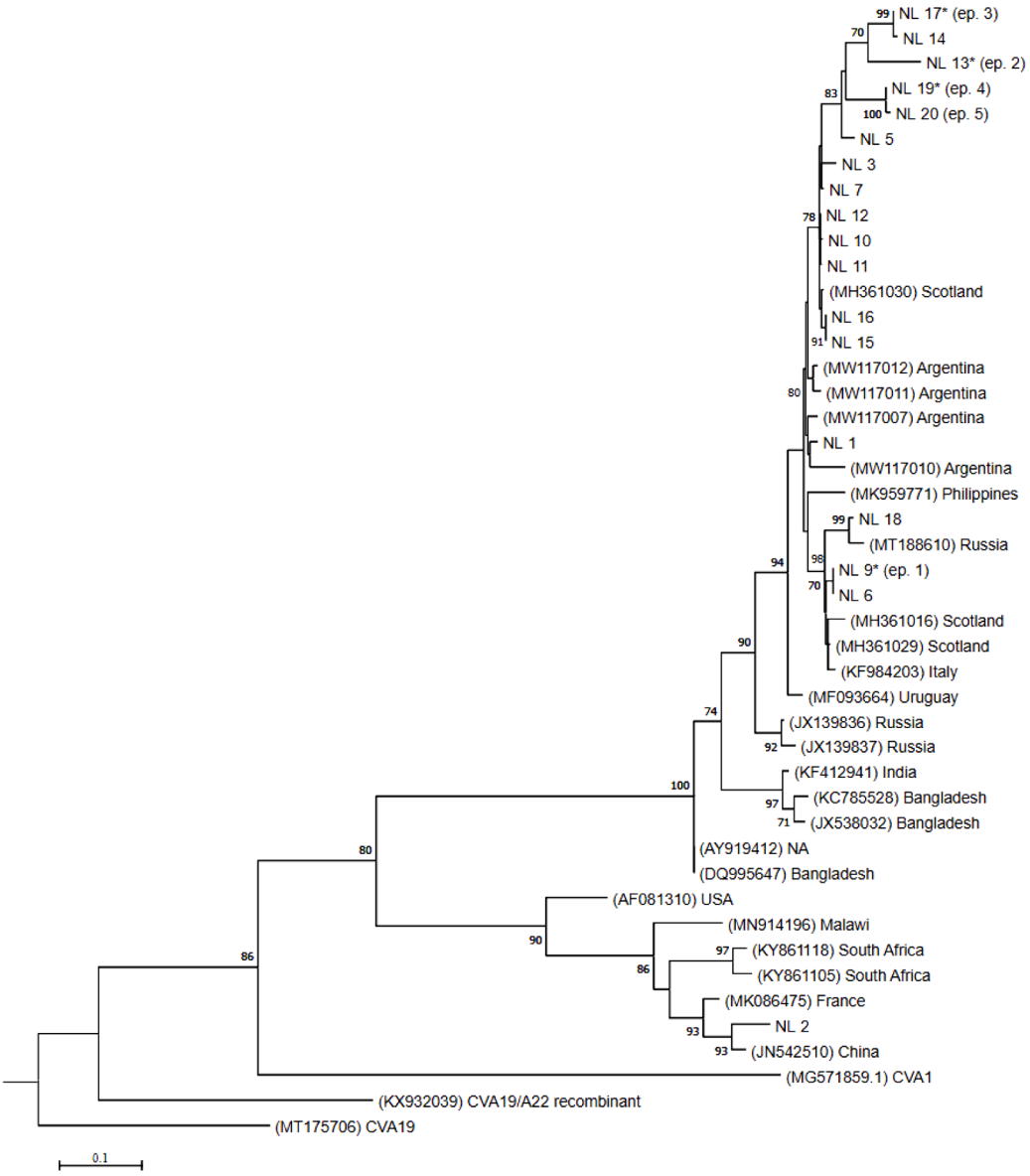

Fig. 1. Phylogenetic analysis of partial VP1 CVA22 sequences. Maximum likelihood trees with 1000 bootstraps were generated. VP1 sequences shorter than 250 bp and highly similar sequences/clusters were trimmed. The tree features eighteen UMCG sequences, three outgroups (CVA1, CVA19 and recombinant CVA19/CVA22 species) and twenty-four reference sequences from GenBank. *CVA22 detection from the patient with the prolonged infection. **Abbreviations:** NA, not available; ep., infection episode from the patient with the prolonged infection.

model (VP1) and a GTR+*G* model (near-complete genomes) using MEGA-11 (Supplementary materials 1.3) [9]. To investigate recombination, bootscan analysis was performed using SimPlot v.3.5.1 (Table S3) [10].

2.4. Ethical and data availability statement

Waivers were obtained by the UMCG Ethics Committee: METc-2018/393 and METc-2021/143. The sequencing data was deposited in the Sequence Read Archive under the BioProject number: PRJNA811787.

3. Results

3.1. Overview of CVA22 at the UMCG

Overall, we detected twenty different CVA22 infection episodes (from sixteen patients) between 2013–2020 (Table 1), with an overall relative frequency of 1.27% out of a total of 1569 enteroviruses (typed using VP1 gene). Using Sanger sequencing, we were able to recover seventeen partial VP1 sequences (from 14 patients). Three samples (NL11, NL14 and NL17) were initially untypeable with Sanger and subsequently processed using NGS to generate two near-complete genomes (Table 2). For the third untypeable sample (Ct 30), we recovered a partial genome, covering the VP1 region. All CVA22 were detected in fecal samples with Ct values ranging from 12–32 from patients between the ages of 1–84 years.

CVA22 was detected at least once per year between 2013–2020, most frequently in October (n=8), with the highest number of recorded detections in October 2018 (n=3) (Fig. S1). We performed phylogenetic analysis of eighteen UMCG CVA22 sequences (partial VP1 gene) (Fig. 1). Phylogenetic analysis revealed that 13/18 UMCG VP1 sequences clustered within the same branch point, closely related to one Scottish strain from wastewater collected in 2015. The remaining five sequences had a high nucleotide similarity to Argentinian (2013) ($n = 1$), Russian (2019) ($n = 1$), Scottish (2015) ($n = 2$) and Chinese (2010) ($n = 1$) CVA22 strains.

3.2. CVA22 whole-genomes

We report the first two near-complete CVA22 genomes from Europe, collected from one lung (2018) and one kidney (2016) transplant recipient presenting with GI symptoms (Table 2). The two obtained

Table 3
Timeline and clinical description of the prolonged CVA22 infection.

Infection episode	Sample name	Collection date	Ct value	Co-detections	Clinical presentation	Diagnosis	Length of stay
1	NL 9	Dec 2015	13	None	Diarrhea, abdominal pain	Gastroenteritis suspected	Outpatient appointment
2	NL 13	May 2018	18	Rhinovirus (RV)	Diarrhea, dyspnea, fever	RV pneumonia	7 days (RV pneumonia)
3	NL 17	Oct 2018	12	None	Diarrhea, abdominal pain	Gastroenteritis suspected	Outpatient appointment
4	NL 19	Feb 2020	15	Influenza A virus H1 (INFA H1)	General malaise, fever, diarrhea, nausea	INFA H1 pneumonia	3 days (INFA H1 pneumonia)
5	NL 20	Oct 2020	16	Sapovirus	Diarrhea, abdominal pain	Gastroenteritis suspected	Outpatient appointment

Abbreviations: RV; rhinovirus, INFA; influenza A virus. Only fecal material was collected.

genomes, NL11 and NL17, were most closely related to a CVA22 virus detected in Bangladesh in 1999 (DQ995647) (90.1% and 89.5% Blastn similarity respectively), which was further confirmed by phylogenetic analysis (Fig. S2, Table S4). Sanger primer-pair annealing analysis revealed high primer miss-matches, particularly at the 3′-end (Table S5). Bootscan analysis indicated no potential recombination events in NL11 or NL17 with other group II complete genomes.

3.3. Prolonged CVA22 infection

We report a possible CVA22 reinfection with three episodes over three years (episodes 1–3; 2015–2018), followed by a prolonged CVA22 infection lasting at least eight months with two additional infection episodes (episodes 4–5; 2020) from an adult lung transplant recipient (Table 3). The enterovirus was suspected to have contributed to GI symptoms and prompted sample collection during an outpatient appointment in three of the five infection episodes (episodes 1, 3, 5), with the remaining suspected to be secondary incidental findings. Comparing the similarities of the partial VP1 sequences ($n = 4$) and near-complete genome ($n = 1$) revealed nucleotide differences of 87.50%−99.38% between infection episodes (Fig. S3).

4. Discussion

Enterovirus typing and surveillance is important to track epidemiological trends and potentially link clinical presentation. In this study, we add to the current literature on CVA22, a relatively understudied enterovirus, and report to the best of our knowledge the first two near-complete genomes from Europe. CVA22 was detected sporadically between 2013–2020 at the UMCG, typically between autumn and winter.

The enormous variations in enterovirus genomes can render genotyping with conventional targeted methods challenging [11–13]. NGS and viral enrichment (for increased sensitivity) was used to complement traditional targeted approaches for genotyping, increase resolution and provide more in-depth phylogenetic analysis. However, currently the limited number of available whole-genome references can impact phylogenetic analysis. This is an important limitation; particularly as grouped sequences do not necessarily come from the same common ancestor.

Although previous studies reporting CVA22 detections have been sparse, there have been some reports detailing GI and neurological presentation, including a patient with diabetes [5,14]. All detections included in this study, of which 80% ($n = 16$) were from adult patients, occurred in fecal samples taken due to GI symptoms. Determining causation or ruling out detections as incidental findings can be challenging, particularly in tertiary hospitals where patients typically have co-morbidities. Enterovirus replication in the intestine is typically asymptomatic. Gastroenteritis caused by enteroviruses is usually mild and resolves within a few weeks, however, in some cases can cause significant morbidity to the patient, particularly if they are immunocompromised [5,15].

One transplant recipient in our study had five different CVA22 infection episodes between 2015–2020. Phylogenetic analysis and sequence comparisons of the VP1 region revealed significant nucleotide changes over five years. While the first three infection episodes appeared to be three separate reinfections, the remaining two episodes appeared to be part of a prolonged infection. It could be that the patient had continued reinfection from a common source (e.g., shared facilities), particularly between the first (2015) and subsequent infection episodes, which were observed in different clusters. It's likely the patients weakened immune system contributed to this persistent infection. At the same time, as the VP1 interacts with the host immune system [16], a high proportion of nucleotide changes are to be expected, which could have also resulted in the failed Sanger sequencing result (e.g., high primer miss-matches), despite repeat testing and low Ct value. As yet, there is limited information on the mutation rate of CVA22, however CV-A enteroviruses are known to be prone to recombination and significant episodic positive selection [17].

CVA22 is frequently found in sewage samples around the world [18–21]. This could indicate that CVA22 is circulating within the community and is likely to be a GI pathogen, owing to its abundance in sewage. The frequent detection of CVA22 in wastewater could indicate that it is asymptomatic in healthy patients (rarely tested in GI screens) and rather becomes detected in severely immunocompromised individuals or patients with underlying conditions (undergoing GI screens more frequently) [5]. We highlight that continual enterovirus surveillance, not only in molecular diagnostic laboratories but also in wastewater and in healthy control patients, is crucial to understand the burden and prevalence of GI pathogens within the community, particularly in patients with underlying conditions.

Funding

H.C. and L.S. received a grant by the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie Actions grant agreement number: 713660-PRONKJEWAIL-H2020-MSCA-COFUND-2015. E.L-F was funded by Quality Control Molecular Diagnostics (QCMD, Glasgow, Scotland) under an unrestricted grant.

Declaration of Competing Interest

All authors declare no conflicts of interest.

Acknowledgements

We want to thank the section of Clinical Virology from the Department of Medical Microbiology and Infection Prevention, University Medical Center Groningen (UMCG) for performing routine diagnostics. We would also like to thank Natacha Couto, John W.A. Rossen and Erwin C. Raangs for expert technical assistance.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcv.2022.105272.

References

[1] Rafal Tokarz, et al., Genomic analysis of coxsackieviruses A1, A19, A22, enteroviruses 113 and 104: viruses representing two clades with distinct tropism within enterovirus C, J. Gen. Virol. 94 (Pt 9) (2013) 1995.

[2] H. de Graaf, E. Pelosi, A. Cooper, J. Pappachan, K. Sykes, I. MacIntosh, D. Gbesemete, T.W. Clark, S.V. Patel, S.N. Faust, M. Tebruegge, Severe enterovirus infections in hospitalized children in the south of England: clinical phenotypes and causative genotypes, Pediatr. Infect. Dis. J. 35 (7) (2016 Jul) 723–727, https://doi.org/10.1097/INF.0000000000001093. PMID: 26882165; PMCID: PMC4985250.

[3] W.A. Nix, M.S. Oberste, M.A. Pallansch, Sensitive, seminested PCR amplification of VP1 sequences for direct identification of all enterovirus serotypes from original clinical specimens, J. Clin. Microbiol. 44 (8) (2006 Aug) 2698–2704, https://doi.org/10.1128/JCM.00542-06. PMID: 16891480; PMCID: PMC1594621.

[4] C.C. Yip, S.K. Lau, P.C. Woo, K.H. Chan, K.Y. Yuen, Complete genome sequence of a coxsackievirus A22 strain in Hong Kong reveals a natural intratypic recombination event, J. Virol. 85 (22) (2011 Nov) 12098–12099, https://doi.org/10.1128/JVI.05944-11. PMID: 22021251; PMCID: PMC3209295.

[5] H. Cassidy, C. van Leer-Buter, H.G.M. Niesters, Enterovirus infections in solid organ transplant recipients: a clinical comparison from a regional university hospital in the Netherlands, Microbiol. Spectr. 10 (1) (2022), e0221521, https://doi.org/10.1128/spectrum.02215-21. Feb 23Epub 2022 Feb 9. PMID: 35138120; PMCID: PMC8826731.

[6] Todd N. Wylie, et al., Enhanced virome sequencing using targeted sequence capture, Genome Res. 25 (12) (2015) 1910–1920.

[7] Leonard Schuele, et al., Application of shotgun metagenomics sequencing and targeted sequence capture to detect circulating porcine viruses in the Dutch–German border region, Transbound. Emerg. Dis. (2021).

[8] Michael Vilsker, et al., Genome Detective: an automated system for virus identification from high-throughput sequencing data, Bioinformatics 35 (5) (2019) 871–873.

[9] S. Kumar, G. Stecher, M. Li, C. Knyaz, K. Tamura, MEGA X: molecular evolutionary genetics analysis across computing platforms, Mol. Biol. Evol. 35 (6) (2018 Jun 1) 1547–1549, https://doi.org/10.1093/molbev/msy096. PMID: 29722887; PMCID: PMC5967553.

[10] K.S. Lole, R.C. Bollinger, R.S. Paranjape, D. Gadkari, S.S. Kulkarni, N.G. Novak, R. Ingersoll, H.W. Sheppard, S.C. Ray, Full-length human immunodeficiency virus type 1 genomes from subtype C-infected seroconverters in India, with evidence of intersubtype recombination, J Virol 73 (1) (1999 Jan) 152–160, https://doi.org/10.1128/JVI.73.1.152-160.1999. PMID: 9847317; PMCID: PMC103818.

[11] C.C. van Leer-Buter, R. Poelman, R. Borger, H.G. Niesters, Newly identified enterovirus c genotypes, identified in the Netherlands through routine sequencing of all enteroviruses detected in clinical materials from 2008 to 2015, J. Clin. Microbiol. 54 (9) (2016 Sep) 2306–2314, https://doi.org/10.1128/JCM.00207-16. Epub 2016 Jun 29. PMID: 27358467; PMCID: PMC5005491.

[12] A. Gelaw, C. Pietsch, Z. Tigabu, U.G. Liebert, Genotyping of enteroviruses and human parechoviruses highlights their diversity in Northwest Ethiopia, J. Med. Virol. (2020 Mar 14), https://doi.org/10.1002/jmv.25765. Epub ahead of print. PMID: 32170868.

[13] L. Posnakoglou, E.B. Tatsi, P. Chatzichristou, T. Siahanidou, C. Kanaka-Gantenbein, V. Syriopoulou, A. Michos, Molecular epidemiology of enterovirus in children with central nervous system infections, Viruses 13 (1) (2021 Jan 13) 100, https://doi.org/10.3390/v13010100. PMID: 33450832; PMCID: PMC7828273.

[14] Sami Oikarinen, et al., Characterisation of enterovirus RNA detected in the pancreas and other specimens of live patients with newly diagnosed type 1 diabetes in the DiViD study, Diabetologia 64 (11) (2021) 2491–2501.

[15] T.R. Townsend, E.A. Bolyard, R.H. Yolken, W.E. Beschorner, C.A. Bishop, W. H. Burns, G.W. Santos, R. Saral, Outbreak of Coxsackie A1 gastroenteritis: a complication of bone-marrow transplantation, Lancet 1 (8276) (1982 Apr 10) 820–823, https://doi.org/10.1016/s0140-6736(82)91872-4. PMID: 6122055.

[16] C.K. Chang, S.R. Wu, Y.C. Chen, K.J. Lee, N.H. Chung, Y.J. Lu, S.L. Yu, C.C. Liu, Y. H. Chow, Mutations in VP1 and 5′-UTR affect enterovirus 71 virulence, Sci. Rep. 8 (1) (2018 Apr 27) 6688, https://doi.org/10.1038/s41598-018-25091-7. Erratum in: Sci Rep. 2018 Jun 4;8(1):8744. PMID: 29703921; PMCID: PMC5923339.

[17] H. Khan, A. Khan, Genome-wide population structure inferences of human coxsackievirus-A; insights the genotypes diversity and evolution, Infect. Genet Evol. 95 (2021 Nov), 105068, https://doi.org/10.1016/j.meegid.2021.105068. Epub 2021 Sep 4. PMID: 34492386.

[18] Manasi Majumdar, Javier Martin, Detection by direct next generation sequencing analysis of emerging enterovirus D68 and C109 strains in an environmental sample from Scotland, Front. Microbiol. 9 (2018) 1956.

[19] H. Harvala, et al., Comparison of diagnostic clinical samples and environmental sampling for enterovirus and parechovirus surveillance in Scotland, 2010 to 2012, Eurosurveillance 19 (15) (2014) 20772.

[20] A. Lizasoain, et al., Human enterovirus diversity by next-generation sequencing analysis in urban sewage samples from buenos aires metropolitan area, Argentina: a retrospective study, Food Environ. Virol. 13 (2) (2021) 259–269.

[21] Maxime Bisseux, et al., Monitoring of enterovirus diversity in wastewater by ultra-deep sequencing: an effective complementary tool for clinical enterovirus surveillance, Water Res. 169 (2020), 115246.