Journal of Clinical Virology 139 (2021) 104845

Contents lists available at ScienceDirect

Journal of Clinical Virology

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

Molecular characterisation and epidemiology of enterovirus-associated aseptic meningitis in the Western and Eastern Cape Provinces, South Africa 2018–2019

Nokwazi Nkosi [a,*], Wolfgang Preiser [a], Gert van Zyl [a], Mathilda Claassen [a], Nadine Cronje [a,b], Jean Maritz [a,b], Howard Newman [a,b], Kerrigan McCarthy [c], Genevie Ntshoe [c,d], Vivien Essel [d], Stephen Korsman [e], Diana Hardie [e], Heidi Smuts [e]

[a] Division of Medical Virology, Department of Pathology, Stellenbosch University and the National Health Laboratory Service Tygerberg, Cape Town, South Africa
[b] PathCare Reference Laboratory, N1 City, Goodwood, Cape Town, South Africa
[c] Division of Public Health, Surveillance and Response, National Institute of Communicable Diseases of the National Health Laboratory Service, Sandringham, Johannesburg, South Africa
[d] School of Health Systems and Public Health, Faculty of Health Sciences, University of Pretoria, Pretoria, South Africa
[e] Division of Medical Virology, Department of Pathology, Faculty of Health Sciences, University of Cape Town, South Africa and the National Health Laboratory Service, Anzio Road, Observatory, Cape Town, South Africa

ARTICLE INFO

Keywords:
Aseptic meningitis
Enterovirus
Echovirus 4
Molecular epidemiology
Western Cape Province
Eastern Cape Province
South Africa

ABSTRACT

Background: Enteroviruses are amongst the most common causes of aseptic meningitis. Between November 2018 and May 2019, an outbreak of enterovirus-associated aseptic meningitis cases was noted in the Western and Eastern Cape Provinces, South Africa.
Objectives: To describe the epidemiology and phylogeography of enterovirus infections during an aseptic meningitis outbreak in the Western and Eastern Cape Provinces of South Africa.
Methods: Cerebrospinal fluid samples from suspected cases were screened using a polymerase chain reaction targeting the 5'UTR. Confirmed enterovirus-associated meningitis samples underwent molecular typing through species–specific VP1/VP2 primers and pan-species VP1 primers.
Results: Between November 2018 and May 2019, 3497 suspected cases of aseptic meningitis were documented in the Western and Eastern Cape Provinces. Median age was 8 years (range 0–61), interquartile range (IQR=4–13 years), 405/735 (55%) male. 742/3497 (21%) cases were laboratory – confirmed enterovirus positive by routine diagnostic PCR targeting the 5'UTR. 128/742 (17%) underwent molecular typing by VP1 gene sequencing. Echovirus 4 (E4) was detected in 102/128 (80%) cases. Echovirus 9 was found in 7%, Coxsackievirus A13 in 3%. 10 genotypes contributed to the remaining 10% of cases. Synonymous mutations were found in most cases, with sporadic amino acid changes in 13 (12.7%) cases.
Conclusion: The aseptic meningitis outbreak was associated with echovirus 4. Stool samples are valuable for molecular typing in CSF confirmed EV-associated aseptic meningitis.

1. Background

The enterovirus (EV) genus in the Picornaviridae family comprises non-enveloped viruses with positive-sense single-stranded ribonucleic acid (RNA) genomes. The predominant route of transmission is faeco-oral or via respiratory droplets.

Following acquisition, some replication occurs in the nasopharynx with spread to the upper respiratory tract lymphatics, however most of the inoculum is ingested. Primary viremia occurs following replication in the lower gastrointestinal tract (GIT) and possibly the nasopharynx with seeding to numerous organ systems including the peripheral nervous system, central nervous system (CNS), liver, lungs and heart [1–3].

Viral entry into the CNS occurs either haematogenously by direct infection of endothelial cells that express EV-permissive receptors and/or through neuronal spread. Within the CNS the virus spreads predominantly through the neuronal route. Extensive local replication can result

* Corresponding author at: Stellenbosch University, Francie van Zijl Drive, Parow, Cape Town, 7505 South Africa.
E-mail addresses: nokwazi@sun.ac.za, nokwazi.nkosi@nhls.ac.za (N. Nkosi).

https://doi.org/10.1016/j.jcv.2021.104845
Received 25 November 2020; Received in revised form 13 April 2021; Accepted 16 April 2021
Available online 24 April 2021

in damage to motor neurons with resulting paralysis [1,2].

Clinical manifestations may develop after an incubation period of 3–21 days and include mild respiratory illness, haemorrhagic conjunctivitis, epidemic pleurodynia, hand-foot- and- mouth disease, meningitis, acute flaccid paralysis/myelitis, encephalitis, sepsis in neonates (hepatitis, encephalitis and myocarditis), and in rare cases death. Severe manifestations are typically described in neonates, persons with B-cell immunodeficiencies and persons on rituximab [3–5].

Aseptic meningitis refers to meningitis with cerebrospinal fluid (CSF) pleocytosis in the absence of a positive Gram stain and culture, without a parameningeal focus or a systemic illness, and with a good clinical outcome [6].

Viruses, particularly EVs, are the leading cause of aseptic meningitis in adults and children worldwide [7–11]. The most common aetiology in U.S. adults hospitalized for meningitis is enteroviruses (50.9%), followed by unknown aetiology (18.7%), bacterial (13.9%), herpes simplex virus (8.3%), non-infectious (3.5%), fungal (2.7%), arboviruses (1.1%), and other viruses (0.8%) [12].

In November 2018, clinicians at a district hospital in the Khayelitsha sub-district of Cape Town, South Africa, reported an increase in the number of aseptic meningitis cases. An increase in EV cases referred for virological diagnosis was also noted in the Southern sub-district and in the largest private sector laboratory serving the Western Cape. A brief review of laboratory data in a similar time period revealed an approximate four times increase compared to the preceding two years from Western Cape laboratories. An increase in cases of aseptic meningitis was also noted by clinicians in the Eastern Cape Province. CSF samples were referred to Tygerberg and Groote Schuur laboratory for molecular testing to confirm EV–associated aseptic meningitis.

Active surveillance of acute flaccid paralysis cases is centralised to the National Institute of Communicable Diseases.

2. Objectives

To describe the epidemiology and phylogeography of EV infections during an aseptic meningitis outbreak in the Western and Eastern Cape Provinces of South Africa.

3. Methods

3.1. Case definitions

A suspected case of aseptic meningitis was defined as any patient presenting with acute onset fever, headache, nausea and vomiting or loss of appetite between November 2018 and May 2019. Presence of neck stiffness, photophobia, irritability or lethargy were supportive symptoms. In addition, CSF lymphocytic predominance, slightly raised protein and no bacterial pathogens identified were regarded as supportive features.

Confirmed cases were defined as patients with EV genome detected in CSF samples by in-house or commercial real time polymerase chain reaction (PCR) targeting the 5′ untranslated region (5′UTR), in whom a bacterial cause was not identified. Testing was done at Tygerberg and Groote Schuur National Health Laboratory Service laboratories and PathCare reference laboratory, South Africa. Eastern Cape Province samples from Dora Nginza Hospital and Settler's Provincial Hospital drainage areas were tested at the Western Cape laboratories. EV-positive samples from all affected areas were sequenced at Tygerberg Virology and Inqaba Biotechnical Industries.

3.2. Laboratory specimens and RNA extraction

CSF samples were collected under aseptic technique in sterile containers and samples were transported and stored at 4 °C.

Total nucleic acid was extracted from 200 µl–500 µl CSF on the automated NucliSens® EasyMag® (Biomerieux, France) or the Qiagen QIAamp MinElute Virus SpinKit® (Hilden, Germany) and eluted into 50 µl elution buffer.

3.3. Screening by RT-PCR of 5′UTR

CSF from suspected cases of aseptic meningitis were screened for EV by amplification of the 5′UTR using the SuperScript One Step RT-PCR System with Platinum Taq DNA Polymerase (Invitrogen) for the outer reaction, followed by a nested reaction, with primers from Zoll et al. (1992). Briefly, 10 µl of RNA was added to 25 µl 2x reaction mix, 1 µl RT/Platinum™ Taq Mix and 50 pmol primers in a 50 µl volume with amplification as per manufacturer's instructions. The nested PCR was performed in a 50 µl reaction containing 2,5 µl outer PCR product, 15 mm Tris-HCL (pH 8), 50 mM KCl, 1.5 mM MgCl2, 0,2 mM dNTPs (ABgene, Epsom, UK), 50 pmol of primers and 1.5 U Supertherm Taq Polymerase (JMR Holdings, Kent, UK). Amplification was as follows: 95 °C for 3 min, 40 cycles of 95 °C for 15 s, 55 °C for 25 s and 72 °C for 35 s followed by 72 °C elongation for 7 min. PCR products were visualised by electrophoresis through a 2% agarose gel, DNA staining and UV illumination. The inner PCR product was 155 bp.

3.4. VP1/2 genotyping of EV-positive samples

Molecular typing of EV-positive samples of sufficient volume was undertaken. Complementary deoxyribonucleic acid (cDNA) synthesis was conducted using the RevertAid™ First strand cDNA Synthesis Kit (Thermo Fisher Scientific™, Waltham, MA, USA). Following cDNA synthesis, nested VP1 or VP2 (EV-C) [13] amplification was undertaken using pan- and species-specific primer sets [14,15]. Five µl was added to the outer master mix as described above (3.3) and amplified for 40 cycles with an outer reaction annealing temperatures of 50 °C and 55 °C for the nested PCR. The VP1 PCR products of EV-A and EV-B were 784 bp and 1085 bp in length respectively, while the EV-C VP2 PCR fragment was 406 bp.[16]

3.5. Sequencing and phylogenetic analysis

PCR products were cleaned up using the Zymoclean Gel DNA Recovery kit (Zymo Research Corp. Irvine, California, USA) and sequenced directly with the BigDye terminator cycle sequencing kit (Applied Biosystems, Foster City CA, USA) using PCR-specific primers. Sequences were confirmed to be of EV origin using BLASTn [17] and the Enterovirus Genotyping Tool Version 0.1 [18]. Sequences from the outbreak and from earlier in 2018 were aligned with reference sequences obtained from the GenBank database, the NIAID Virus Pathogen Database and Analysis Resource (ViPR) [19] through the website http://www.viprbrc.org and Echovirus 4 (E4) sequences from an earlier outbreak in 2010/2011 in Tshwane (Gauteng Province, South Africa) [20] using BioEdit version 7.2.5 [21]. Neighbour-joining phylogenetic trees were constructed in MEGA 6.06 using the maximum-likelihood algorithm and Tamura-Nei model with 1000 bootstrap re-samplings [22]. A Highlighter plot was generated by Highlighter [23] (www.hiv.lanl.gov) using VP1 aligned nucleotide and amino acid sequences to visualize individual sequence polymorphisms [23] .

3.6. Patient data collection

Patient demographic data was retrieved from the National Health Laboratory Service (NHLS) laboratory information system (LIS) and the private sector laboratories in the Western Cape Province.

3.7. Public health response

The Western Cape Provincial Communicable Disease Control (CDC) was notified. A line list of probable, suspected and confirmed cases compiled by the Western Cape CDC, containing demographic details,

clinical details, residential address and laboratory test results, suggested disseminated community involvement. Engagement was undertaken with various stakeholders, namely clinicians within the Western and Eastern Cape Provinces, the City of Cape Town Environmental Health Department and community radio stations. These events occurred within a background of a prolonged drought period in both provinces and media reports of alleged effluent discharge from the Zandvliet wastewater treatment plant in the Western Cape Province contaminating the Kuilsrivier.

Mobile clinics and community health care workers were dispatched, community awareness campaigns undertaken through use of local radio stations and information pamphlets, hand hygiene measures were encouraged, and communities advised to avoid interaction with the river.

4. Results

4.1. The outbreak

Between November 2018 and May 2019, 3497 cases of suspected aseptic meningitis were detected in Western and Eastern Cape Provinces. 742 (21%) were laboratory confirmed as EV-associated aseptic meningitis cases by PCR targeting the EV 5′UTR. Of these, 80% (594/742) were from the Western Cape Province. The epidemiological curve is shown in Fig. 1. A notable increase in number of EV-positive cases was observed from November 2018 onward. Additional cases continued to be reported with a peak occurring in February 2019 before gradually declining to low levels towards end of May 2019 (Fig. 1). Cases were reported from several health districts, with cases from Khayelitsha 105/742 (14%), Mitchell's Plain 116/742 (16%) and Tygerberg sub-districts (6%) accounting for the largest proportion of cases reported.

Median age of EV-positive confirmed cases was 8 years (range 0–61), interquartile range (IQR = 4–13 years) and 405/735 (55%) male.

4.2. Molecular epidemiology

Of 742 cases testing positive for EV, 128 (17%) cases underwent molecular typing by VP1/VP2 gene amplification. E4 was detected in 102 (80%) of cases sequenced. Echovirus 9 was found in 9 cases (7%), Coxsackievirus A13 in 4 (3%), and there were two cases each (1.6%) of Echovirus 7, 18 and 30 and one case each (0.8%) of Coxsackievirus B3, B5, Echovirus 6,32,33, Enterovirus A71 and Enterovirus B74.

Nucleotide sequence alignment of the VP1 region showed a pairwise identity of outbreak strains ranging from 95.7 – 100%. The similarity is visualized by Highlighter Plot using the earliest outbreak virus dated 26th November 2018 as comparator (Fig. 2). There appeared to be a trend to the increasing presence of nt 180T over time, with 9/38 (23.7%) of samples from November 2018- January 2019 showing this nucleotide, which increased to 26/45 (57.8%) over the remaining period of the outbreak. All 18 samples from Khayelitsha showed a C at this position. The majority of nucleotide changes, including the aforementioned, were synonymous, with only sporadic amino acid changes found in 13 (12.7%) cases and none were located in the neutralizing-epitope BC-loop of VP1 (data not shown). A BLASTn search identified the closest homology (92.0 – 94.5%) of the outbreak strain E4 with three E4 strains from India (JN203683-JN203685).

VP1 gene phylogenetic analysis revealed a monophyletic cluster of the Western and Eastern Cape E4 sequences with a high bootstrap value (97%) (Fig. 3). The Khayelitsha samples clustered predominantly at the base of tree indicating possible source of epidemic. The samples from the Eastern Cape (n = 4) formed a separate clade with other samples from Western Cape health sub-districts and were characterised by nucleotides 229T and 231A in the VP1 gene (Fig. 2). A previously detected and sequenced E4 case from July 2018 did not cluster with the outbreak virus. After the epidemic had ended, an E4-positive sample taken 13th May 2019 grouped with this former virus.

5. Discussion

We describe an outbreak of enterovirus-associated aseptic meningitis across two South African provinces between November 2018 and May 2019, characterized by E4 predominance. This is the second largest E4 outbreak described in the Western Cape Province after an outbreak published by McIntyre and Keen, 1993 [24].

E4 was the predominant type in samples successfully sequenced (80%). Local circulation of E4 in the Western Cape Province was previously documented in 1981 and 1986 with 1205 cases [24]. Prior to this epidemic, an outbreak of E4 had most recently been described in Tshwane, 2010–2011, in a cluster of 24 cases [20]. However, the Tshwane 2010–2011 sequences formed a separate clade to the Western and Eastern Cape sequences with a bootstrap support value of 71%. The Tshwane outbreak sequences were in turn most closely related to sequences from India.

Viral factors facilitating spread of EV infections have been well described [1,25]. Shedding in asymptomatic patients, constituting the majority of EV cases, close contact and poor hygiene are additional factors. Climactic factors are postulated to contribute towards the seasonal distribution. The clustering and basal location of the Khayelitsha

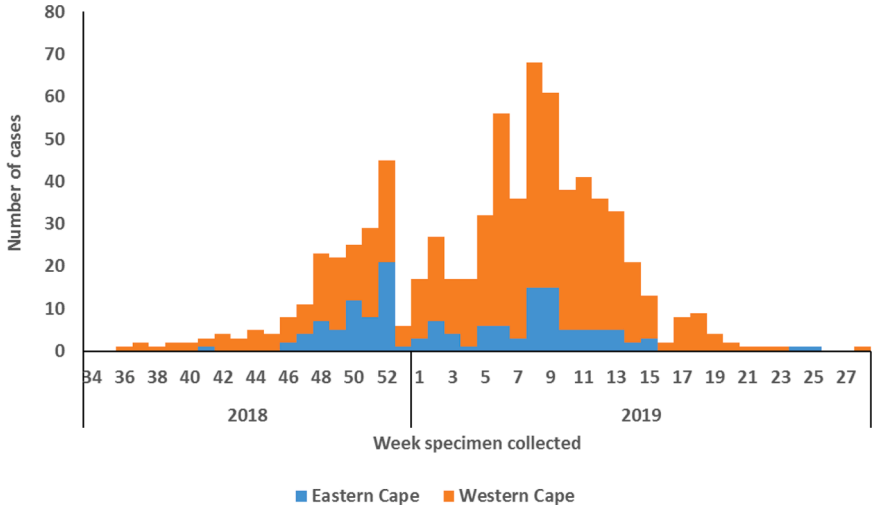

Fig. 1. Epidemic curve of EV-associated Aseptic Meningitis cases by week specimen collected, Western Cape (N = 594) and Eastern Cape (N = 148) provinces, South Africa.

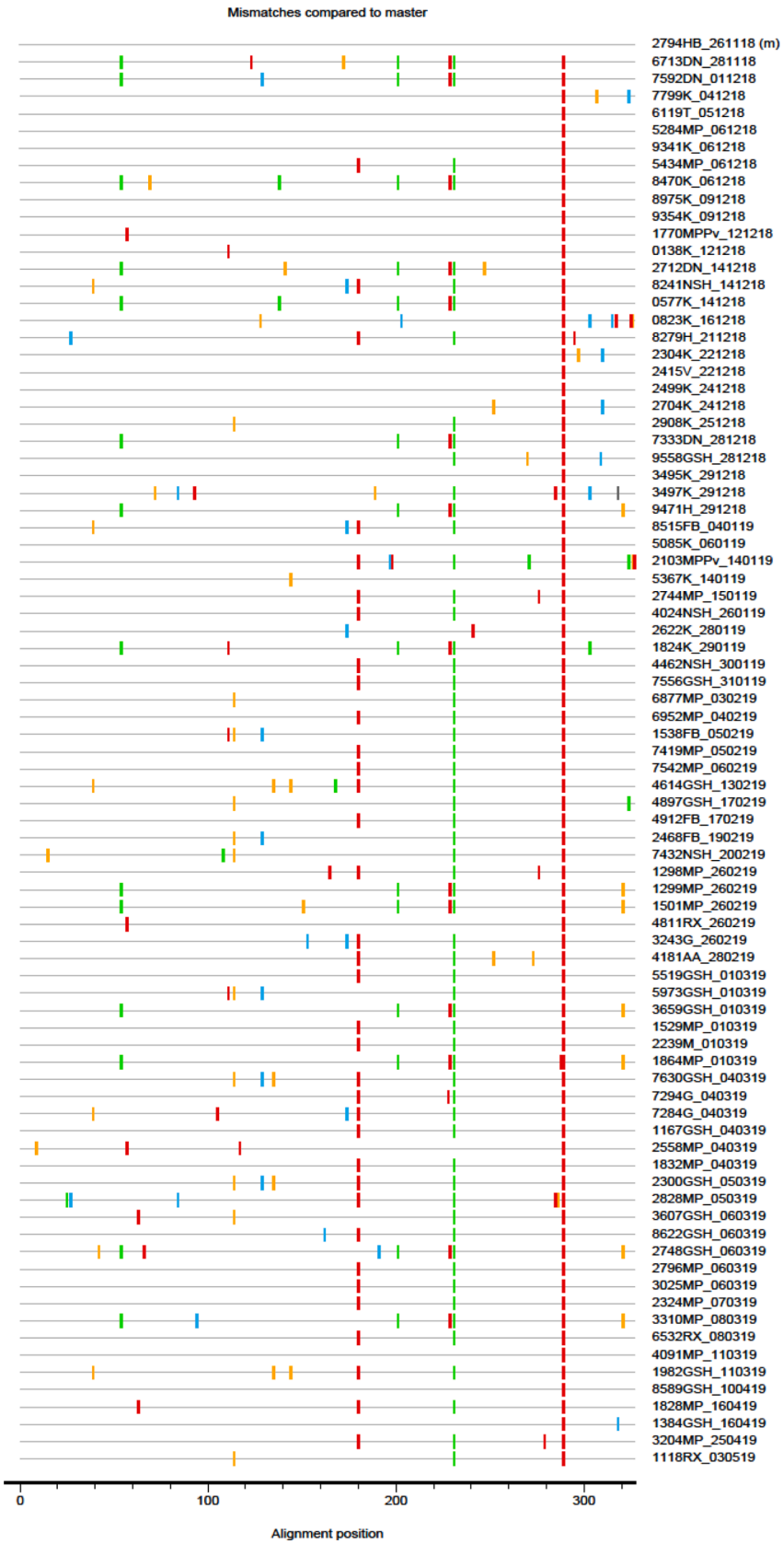

Fig. 2. Nucleotide highlighter plot of Echovirus 4 sequences in the Western Cape and Eastern Cape Provinces arranged by date of sample.

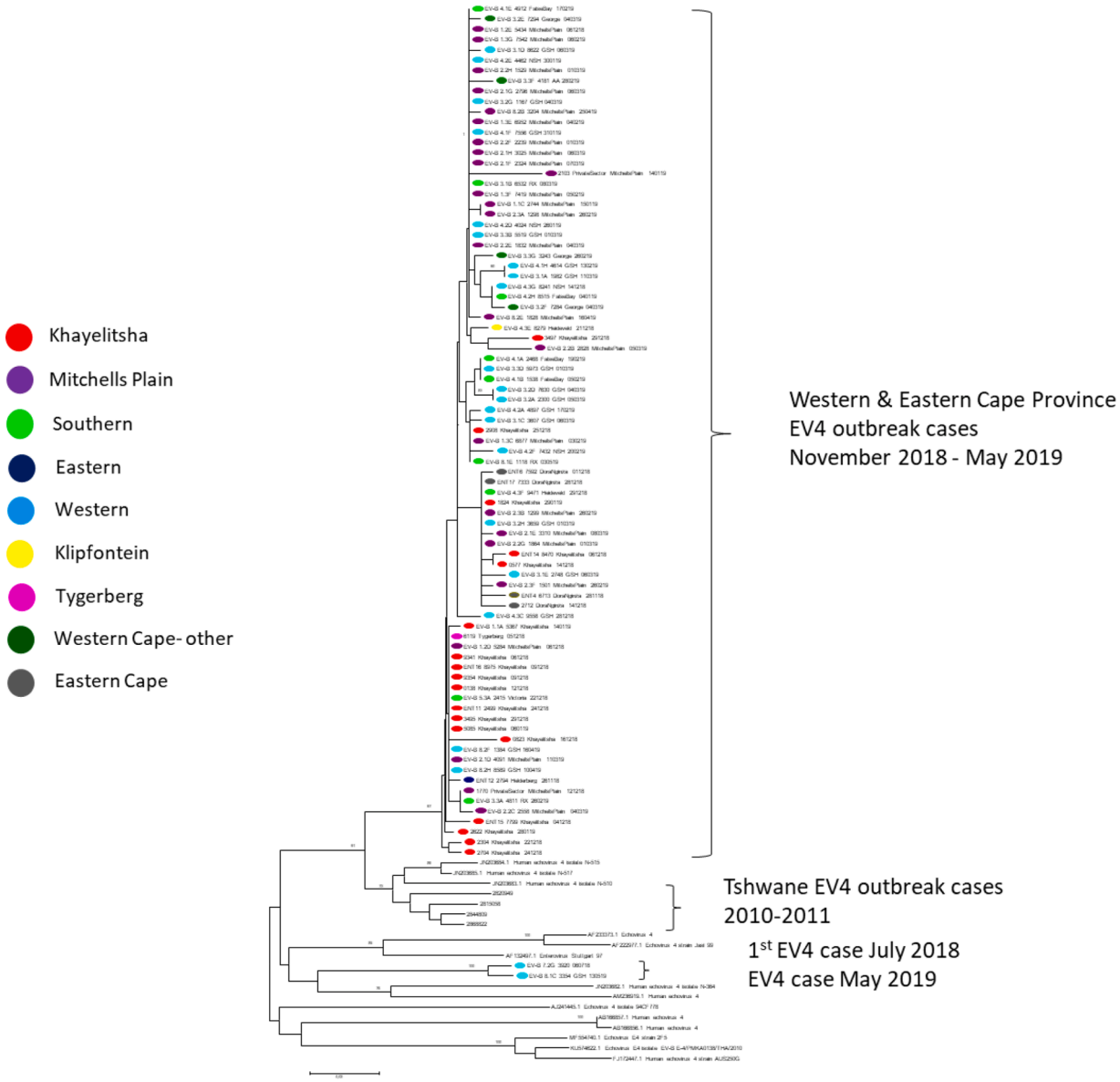

Fig. 3. Maximum Likelihood phylogenetic tree of Echovirus 4 sequences, with 1000 bootstrapping resampling. Each Cape Town Metro health sub-district and Eastern Cape samples in different colour. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

sequences suggest that these may have been the founder viruses in this outbreak (Fig. 3).

That the December peak in the Eastern Cape Province was accompanied by a decline in cases in the Western Cape Province, during the holiday season provides an opportunity to infer on the role of host migration patterns as a contributor towards the geographical spread of viral infections. The subsequent Western Cape Province resurgence in January, coincided with the end of the holiday season (Fig. 1).

Co-circulation of multiple EV variants is frequent within a season, with differences in endemicity and potential for pandemicity. Different interseasonal patterns have been described [25,26]. Echovirus 9 (E9) was the second most frequently identified type during this time period. Circulation of multiple EV variants differs temporally and geographically; and may be affected by various factors such as viral evolution and recombination events, population bottlenecks, virulence, host immunity and introduction of novel types [25]. Species B EV are the commonest cause of aseptic meningitis [27–29]. Similarly, within our study, the majority of types detected were within Species B.

Limitations of our study include lack of longitudinal data of EV types circulating outside of this time period to generate further comparisons on longitudinal type predominance and potential molecular evolution events, in the absence of routine EV surveillance programmes. Not all samples testing EV positive on screening could be typed due to insufficient sample availability and it therefore is not certain if the sequenced samples are fully representative of the outbreak.

E4 was detected in 19/22 (86%) stool samples from patients with confirmed EV aseptic meningitis on CSF samples. Viral concentrations are often higher in stool than in CSF samples.

Molecular typing of EVs retains utility in defining the epidemiology of circulating EV strains by laboratories as part of active/ passive surveillance measures, but also during periods of EV-associated disease clusters and outbreaks.

GenBank accession numbers

Accession numbers MW216583-MW216665.

Financial support statement

This work was supported by the National Health Laboratory Service Research Trust Development Grant (94762) and the Poliomyelitis Research Foundation (PRF15/28).

CRediT authorship contribution statement

Nokwazi Nkosi: Funding acquisition, Investigation, Methodology, Data curation, Writing - original draft, Writing - review & editing. **Wolfgang Preiser:** Conceptualization, Funding acquisition, Writing - review & editing. **Gert van Zyl:** Conceptualization, Investigation, Methodology, Writing - review & editing. **Mathilda Claassen:** Investigation, Methodology, Data curation, Data curation. **Nadine Cronje:** Investigation, Methodology, Data curation. **Jean Maritz:** Investigation, Methodology, Writing - review & editing. **Howard Newman:** Investigation, Methodology, Writing - review & editing. **Kerrigan McCarthy:** Writing - review & editing. **Genevie Ntshoe:** Data curation, Writing - review & editing. **Vivien Essel:** Data curation. **Stephen Korsman:** Data curation, Writing - review & editing. **Diana Hardie:** Writing - review & editing. **Heidi Smuts:** Funding acquisition, Investigation, Methodology, Data curation, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

We have no conflict of interest to declare.

Acknowledgements

We acknowledge the Virology staff of Tygerberg and Groote Schuur National Health Laboratory Service Virology laboratories. We thank Wakeel Mathure, Helena Rabie (Department of Paediatrics and Child Health Tygerberg Hospital and Stellenbosch University), clinicians at Khayelitsha District Hospital, Tygerberg Hospital and Grahamstown Hospital as well as Charlene Jacobs, the Western Cape CDC, public health response team, Mahlatse Rapholo and NICD public health surveillance and response. We thank Maureen Taylor and Marianne Wolfaardt for the provision of historical Tshwane Echovirus 4 sequences.

References

[1] J. Romero, Enteroviruses, in: D. Richman, R. Whitley, F. Hayden (Eds.), Clinical Virology. Third Edit, eds, ASM Press, 2009, pp. 1031–1061.
[2] P. Minor, P. Muir, Enteroviruses, in: A. Zuckerman, J. Banatvala, B. Schoub, P. Griffiths, P. Mortimer (Eds.), Principles and Practice of Clinical Virology. Sixth Edit, eds, Wiley Blackwell, 2009, pp. 601–624.
[3] H. Harvala, J. Calvert, D. Van Nguyen, et al., Comparison of diagnostic clinical samples and environmental sampling for enterovirus and parechovirus surveillance in Scotland, 2010 to 2012, Euro Surveill. Bull. Eur. sur les Mal. Transm. = Eur. Commun. Dis. Bull. 19 (15) (2014).
[4] H. Rudolph, H. Schroten, T. Tenenbaum, Enterovirus infections of the central nervous system in children: an update, Pediatr. Infect. Dis. J. (2016), https://doi.org/10.1097/INF.0000000000001090.
[5] D. Antona, M. Kossorotoff, I. Schuffenecker, et al., Severe paediatric conditions linked with EV-A71 and EV-D68, France, May to October 2016, Euro Surveill. Bull. Eur. sur les Mal. Transm. = Eur. Commun. Dis. Bull. 21 (46) (2016), https://doi.org/10.2807/1560-7917.ES.2016.21.46.30402.
[6] B. Shukla, E.A. Aguilera, L. Salazar, S.H. Wootton, Q. Kaewpoowat, R. Hasbun, Aseptic meningitis in adults and children: diagnostic and management challenges, J. Clin. Virol. (2017), https://doi.org/10.1016/j.jcv.2017.07.016.
[7] B.E. Lee, H.D. Davies, Aseptic meningitis, Curr. Opin. Infect. Dis. 20 (3) (2007) 272–277, https://doi.org/10.1097/QCO.0b013e3280ad4672.
[8] M. Kaminski, V. Grummel, D. Hoffmann, A. Berthele, B. Hemmer, The spectrum of aseptic central nervous system infections in southern Germany – demographic, clinical and laboratory findings, Eur. J. Neurol. (2017), https://doi.org/10.1111/ene.13335.
[9] F. McGill, M.J. Griffiths, L.J. Bonnett, et al., Incidence, aetiology, and sequelae of viral meningitis in UK adults: a multicentre prospective observational cohort study, Lancet Infect. Dis. (2018), https://doi.org/10.1016/S1473-3099(18)30245-7.
[10] P. Chen, X. Lin, G. Liu, et al., Analysis of enterovirus types in patients with symptoms of aseptic meningitis in 2014 in Shandong, China, Virology (2018), https://doi.org/10.1016/j.virol.2018.01.022.
[11] N. Liu, L. Jia, J. Yin, et al., An outbreak of aseptic meningitis caused by a distinct lineage of coxsackievirus B5 in China, Int. J. Infect. Dis. (2014), https://doi.org/10.1016/j.ijid.2014.02.005.
[12] R. Hasbun, N. Rosenthal, J.M. Balada-Llasat, et al., Epidemiology of Meningitis and Encephalitis in the United States, 2011-2014, Clin. Infect. Dis. 65 (3) (2017) 359–363, https://doi.org/10.1093/cid/cix319.
[13] N.J. McLeish, J. Witteveldt, L. Clasper, et al., Development and assay of RNA transcripts of enterovirus species A to D, rhinovirus species a to C, and human parechovirus: assessment of assay sensitivity and specificity of real-time screening and typing methods, J. Clin. Microbiol. 50 (9) (2012) 2910–2917, https://doi.org/10.1128/JCM.01172-12.
[14] E.C.M. Leitch, H. Harvala, I. Robertson, I. Ubillos, K. Templeton, P. Simmonds, Direct identification of human enterovirus serotypes in cerebrospinal fluid by amplification and sequencing of the VP1 region, J. Clin. Virol. 44 (2) (2009) 119–124, https://doi.org/10.1016/j.jcv.2008.11.015.
[15] 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) 2698–2704, https://doi.org/10.1128/JCM.00542-06.
[16] H. Smuts, S. Cronje, J. Thomas, D. Brink, S. Korsman, D. Hardie, Molecular characterization of an outbreak of enterovirus-associated meningitis in Mossel Bay, South Africa, December 2015-January 2016, BMC Infect. Dis. 18 (1) (2018) 709, https://doi.org/10.1186/s12879-018-3641-4.
[17] Basic Local Alignment Search Tool. https://blast.ncbi.nlm.nih.gov/Blast.cgi. Accessed 15 January 2018.
[18] Enterovirus Genotyping Tool Version 0.1. http://www.rivm.nl/mpf/enterovirus/typingtool. Accessed 20 November 2017.
[19] B.E. Pickett, E.L. Sadat, Y. Zhang, J.M. Noronha, R.B. Squires, V. Hunt, et al., ViPR: an open bioinformatics database and analysis resource for virology research, Nucleic. Acids. Res. 40 (2012) (Database issue):D593-598.
[20] M. Wolfaardt, A. Büchner, M. Myburgh, T. Avenant, N.M. du Plessis, M.B. Taylor, Molecular characterisation of enteroviruses and clinical findings from a cluster of paediatric viral meningitis cases in Tshwane, South Africa 2010–2011, J. Clin. Virol. 61 (3) (2014) 400–405, https://doi.org/10.1016/j.jcv.2014.08.001.
[21] T.A. Hall, BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT, Nucl. Acids Symp. Ser. 41 (1999) 95–98.
[22] K. Tamura, G. Stecher, D. Peterson, A.K.S Filipski, MEGA6: molecular evolutionary genetics analysis version 6.0, Mol. Biol. Evol. 30 (12) (2013) 2725–2729.
[23] HIV Database Tools. https://www.hiv.lanl.gov/content/sequence/HIV/HIVTools.html. Accessed 5th December 2019.
[24] J.P. McIntyre, G.A. Keen, Laboratory surveillance of viral meningitis by examination of cerebrospinal fluid in Cape Town, 1981-9, Epidemiol. Infect. 111 (2) (1993) 357–371, https://doi.org/10.1017/s095026880005706x.
[25] A.N. Lukashev, Y.A. Vakulenko, N.A. Turbabina, A.A. Deviatkin, J.F. Drexler, Molecular epidemiology and phylogenetics of human enteroviruses: is there a forest behind the trees? Rev. Med. Virol. 28 (6) (2018) e2002, https://doi.org/10.1002/rmv.2002.
[26] M. Pons-Salort, M.S. Oberste, M.A. Pallansch, et al., The seasonality of nonpolio enteroviruses in the United States: patterns and drivers, Proc. Natl Acad. Sci. 115 (12) (2018) 3078, https://doi.org/10.1073/pnas.1721159115. LP - 3083.
[27] J. Richter, C. Tryfonos, C. Christodoulou, Molecular epidemiology of enteroviruses in Cyprus 2008-2017, PLoS ONE 14 (8) (2019), https://doi.org/10.1371/journal.pone.0220938 e0220938.
[28] E. Ramalho, I. Sousa Jr, F. Burlandy, et al., Identification and phylogenetic characterization of human enteroviruses isolated from cases of aseptic meningitis in Brazil, 2013-2017, Viruses 11 (8) (2019) 690, https://doi.org/10.3390/v11080690.
[29] S. Suresh, W.D. Rawlinson, P.I. Andrews, Stelzer-Braid S. Global epidemiology of nonpolio enteroviruses causing severe neurological complications: a systematic review and meta-analysis, Rev. Med. Virol. 30 (1) (2020) e2082, https://doi.org/10.1002/rmv.2082.