Predominance of enterovirus B and echovirus 30 as cause of viral meningitis in a UK population

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Abstract

Background/Objectives: Enteroviruses are the most common cause of aseptic or lymphocytic meningitis, particularly in children. With reports of unusually severe neurological disease in some patients infected with enterovirus D68 in North America, and a recent increase in the number of paediatric enterovirus meningitis cases presenting in this UK Midlands population, a local surveillance study was performed.

Study design: Cerebrospinal fluid (CSF) samples received were tested using the polymerase chain reaction (PCR) for HSV-1/2, VZV, enteroviruses and parechoviruses. Enterovirus PCR positive CSF samples were sent for further serotyping. A phylogenetic tree was constructed of the echovirus 30 VP1 sequences, where sufficient sample remained for sequencing.

Results: The number of enterovirus positive CSFs from each year were: 21 (2008), 7 (2011), 53 (2012), 58 (2013) and 31 (2014). Overall, 163 of the 170 serotyped enteroviruses belonged to the species B (echovirus 5, 6, 7, 9, 11, 13, 16, 17, 18, 21, 25, 30; coxsackie B1, B2, B3, B4, B5, A9) , with only 7 belonging to species A (coxsackie A2, A6, A16 and enterovirus 71). Echovirus 30 was the predominant serotype overall, identified in 43 (25.3%) of samples, with a significantly higher proportion in the adult age group (37.3%) compared to the infant age group (12.3%). Phylogenetic analysis showed that these UK Midlands echovirus 30 VP1 sequences clustered most closely with those from Europe and China.

Conclusion: This small local study showed a continued predominance of echovirus 30 as a cause of viral meningitis, particularly in adults, though more surveillance is needed.

35 ***Short Communication***

36

37 **Background/Objectives**

38 Viral meningitis is usually a self-limiting disease, affecting all ages, typically
39 presenting with fever, headache, neck stiffness, photophobia, nausea and vomiting.
40 Enteroviruses (EVs) are the most common cause of such aseptic or lymphocytic meningitis,
41 particularly in children. Various studies over several decades have shown that enteroviruses
42 are the predominant causal agents for this disease, with echovirus 30 being one of the
43 commonest (**Oberste et al. 1999; Brunel et al. 2008; Croker et al. 2015**).

44 With the appearance of unusually severe neurological disease in patients infected
45 with EV D68 in North America in the past year (**Ayscue et al. 2014; Greninger et al. 2015**),
46 and most recently in Norway (**Pfeiffer et al. 2015**), there has been a recent surge of interest
47 in the epidemiology and clinical spectrum of diseases associated with this EV D68 serotype
48 (**Poelman et al. 2015; Farrell et al. 2015**).

49 In the UK, the use of PCR-based diagnostic testing for the detection of enteroviruses
50 has been increasing in recent years (**Kadambari et al. 2014**). In this small retrospective
51 study, we present results obtained from the routine diagnostic testing of cerebrospinal fluid
52 (CSF) samples collected from patients (mainly children) presenting with symptoms of
53 meningitis (fever, headache, generalised sepsis, malaise, and photophobia and neck
54 stiffness, where described).

55 The aim was to characterise the enterovirus strains circulating among the UK
56 Midlands (Birmingham and Leicester) population between 2008 and 2014.

57

58 **Study design**

59 Cerebrospinal fluid samples collected by clinicians (neonatal, paediatric, adult,
60 neurology, etc.) according to their local clinical protocols were sent for the investigation of
61 meningitis cases. Each sample was tested using real-time polymerase chain reaction (PCR)
62 assays for: herpes simplex viruses 1 and 2 (HSV 1/2), varicella zoster virus (VZV), using in-

house real-time PCR assay; and enteroviruses and parechoviruses using a commercial assay (the FTD EPA kit, Fast-Track diagnostics Ltd., Sliema, Malta), according to manufacturer's instructions. The enterovirus PCR positive CSF samples (all of which were negative HSV 1/2, VZV and parechoviruses) were sent for further confirmation and serotyping at the National Reference Laboratory (Public Health England, Colindale, UK). Serotyping was based on the sequencing of a short (~360 bp) region of VP1 (**Iturriza-Gómara et al. 2006**). The enterovirus serotype results for 2008 (data from 2009-2010 was not available), 2011-2014 was summarised, tabulated, and analysed by age and year of detection.

Results

The number of enterovirus positive CSFs from each year (by collection date) were: 21 (2008), 7 (2011), 53 (2012), 58 (2013) and 31 (2014). Overall, 163 of the 170 samples sent for serotyping belonged to species B enteroviruses (echovirus 5, 6, 7, 9, 11, 13, 16, 17, 18, 21, 25, 30; coxsackie B1, B2, B3, B4, B5, A9) , with only 7 belonging to species A enteroviruses (coxsackie A2, A6, A16 and enterovirus 71). Echovirus 30 was the predominant serotype, overall, identified in 43 (25.3%) of samples, with a significantly higher proportion in the adult age group (37.3%) compared to the infant age group (12.3%, z-score; p value <0.001). Echovirus 30 also showed an epidemic pattern, being the dominant serotype in 2008 and 2012, but relatively less common in the other years (**Fig. 1**).

Where the diagnostic PCR products from echovirus 30 positive CSF samples could be sequenced successfully (9 from Birmingham and 7 from Leicester), these VP1 partial sequences (final length 273 bp) were aligned and manually edited to construct a maximum likelihood tree (**Fig. 2**) (GenBank Accession nos.: KU645936-KU645951). This shows that the older 2008 Birmingham samples cluster closely with echovirus 30 VP1 sequences mostly from France (8 out of 9), but also with one from Greece (1 out of 9). The more recent 2014 Leicester sequences (in blue font in the online PDF version) cluster closely mostly with

echovirus 30 VP1 sequences from Italy (5 out of 7), but also with some from China (2 out of 7).

Discussion

The most striking findings in this study were the continued predominance of the enterovirus B species, particularly echovirus 30, as causes of viral meningitis and the marked predilection for the infant and adult age groups. This is consistent with other recent studies (**Xiao et al. 2013; Milia et al. 2013; Croker et al. 2015**).

There was a notable strong age bias, which was likely the result of local clinical assessment and sampling protocols, with 82/170 (48.2%) of cases in infants (i.e. under 1-year old), 75/170 (44.1%) in adults (18 years and older), and only 13/170 (7.6%) in the 1-17 age group. So, either few 1-17 year olds were infected, or if they were infected they did not become ill enough to present to healthcare services.

For the infant and adult populations, the large increase in the numbers of positive cases in 2012 (predominantly: coxsackie B1 in infants; echovirus 6 in adults) and 2013 (predominantly: coxsackie B3 in infants; echovirus 30 in adults) may have been partly due to greater access to the enterovirus serotyping service which started in 2012. The number of serotyped infections was maintained in the infant age group during 2014 (predominantly echovirus 7/coxsackie B5), but decreased in adults (predominantly echovirus 30).

In summary, this a representative picture of the enteroviruses circulating in this patient population, causing viral meningitis and/or sepsis of sufficient severity to warrant CSF collection for diagnostic testing. In particular, within the predominant enterovirus serotype, echovirus 30, there was surprising genetic diversity, with the VP1 sequences being similar to those obtained from Europe and China. This provides a reservoir from which novel strains may potentially emerge, perhaps as an outbreak event.

More thorough, systematic surveillance, covering a wider population, is needed to understand the epidemiology of this disease. However,this can only be performed using CSF

samples on the basis of clinical need. This is due to the invasive nature of the lumbar puncture procedure that is required to obtain the CSF for testing, which would be difficult to justify in less ill individuals.

Conflict of Interest Declaration

None of the authors have any conflicts of interest to declare.

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Figure legends

Fig 1. Enterovirus (EV) serotype distributions for patients (infants, children and adults) presenting with viral meningitis CB – Coxsackie B virus, E – echovirus, with the numbers representing individual serotypes. 'Other' represents other less common EV serotypes that are not shown individually (Coxsackie A2, A6, A9, A16, B2, Echovirus 5, 13, 16, 17, 21, 25, Enterovirus 71).

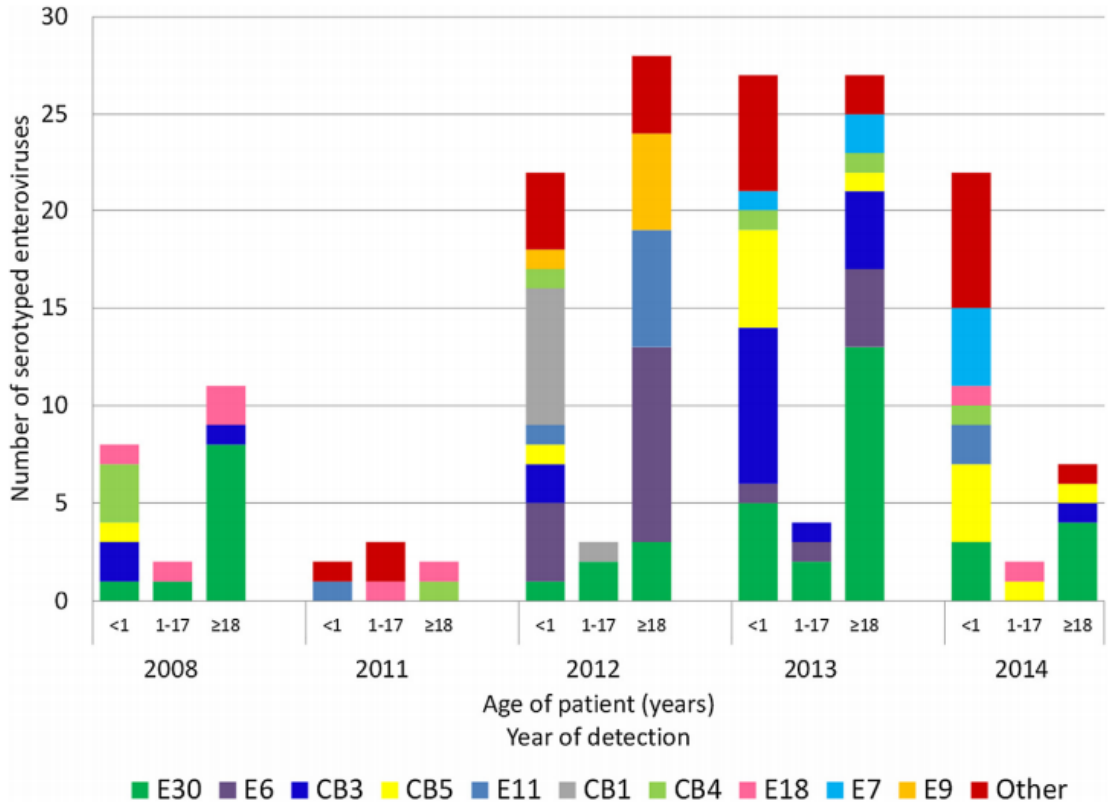

Fig. 2. Maximum likelihood phylogenetic tree, mid-point-rooted, showing the clustering of these UK Midlands CSF echovirus 30 VP1 partial gene sequences (273 bp long) (9 from Birmingham, 2008 and 7 from Leicester, 2014), with closely related sequences downloaded from Genbank. The cluster pattern can be clearly resolved into a European (fine dotted line box) branch and a China (dashed-dotted line box) branch. The numbers on the branches indicate the value the Shimodaira-Hasegawa (SH) support statistic for that branch, as implemented in Fast Tree 2.1.7 (http://meta.microbesonline.org/fasttree/)