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Short communication

An enhanced Enterovirus surveillance system allows identification and characterization of rare and emerging respiratory enteroviruses in Denmark, 2015–16

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ABSTRACT

Background: The potential for outbreaks due to Enteroviruses (EV) with respiratory tropism, such as EV-D68, and the detection of new and rare EV species C is a concern. These EVs are typically not detected in stool specimens and may therefore be missed by standard EV surveillance systems. Following the North American outbreak of EV-D68 in 2014, Denmark piloted an enhanced EV surveillance system that included the screening of respiratory samples.

Objectives: We aim to report clinical manifestations and phylogenetic descriptions from the rare and emerging EVs identified thereby demonstrating the usefulness of this system.

Study design: Positive EV samples received through the enhanced non-polio EV pilot surveillance system were characterized by sequencing fragments of VP1, VP2 and VP4 capsid proteins and clinical observations were compiled.

Results: Between January 2015 and October 2016, six cases of rare genotypes EV-C104, C105 and C109 and nine cases of EV-D68 were identified. Patients presented with mild to moderately severe respiratory illness; no paralysis occurred. Distinct EV-C104, EV-C109 and EV-D68 sequences argue against a common source of introduction of these genotypes in the Danish population.

Conclusions: The enhanced EV surveillance system enabled detection and characterization of rare EVs in Denmark. In order to improve our knowledge of and our preparedness against emerging EVs, public health laboratories should consider expanding their EV surveillance system to include respiratory specimens.

1. Background

In 2014, an outbreak of severe respiratory illness and neurological impairment affected North America. Enterovirus (EV) D68 was identified in more than 1100 cases (mostly children), and in specimens from 14 patients who died in the U.S. in 2014 [1]. The potential for similar outbreaks due to EV-D68 or other respiratory EVs is a concern. Since 2009, new EV species C have emerged [2–6], with a wide range of clinical presentations reported, including lower and upper tract respiratory infections but also polio-like symptoms such as acute flaccid myelitis and paralysis [7], fatal in one case from Democratic Republic of Congo [3]. Recently, detection of EV-C104, C105, C109 and C117 in respiratory samples of mild to moderately severe cases was reported in the Netherlands indicating that these genotypes are also circulating in northern Europe [8]. An outbreak of EV-D68 new lineage B3 was also reported from Sweden over the summer 2016, with over 70 cases identified leading to severe respiratory or neurological symptoms, or death, in 11 patients [9].

It is well known that several of the rare and emerging respiratory EVs are usually not found in stools [8]. This is likely explained by varying physio-chemical characteristics, which makes some of these rare EVs susceptible to gastric acid and difficult to detect in stools. They

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are therefore missed by the standard EV surveillance systems, which were primarily designed to capture cases of poliovirus-associated flaccid paralysis and meningitis. In Denmark, an enhanced non-polio EV pilot surveillance system was implemented in September 2014, immediately after the recognition of the U.S. outbreak of EV-D68, to include respiratory samples [10].

2. Objectives

Our objectives were to report emerging EVs circulating in Denmark and identified through the enhanced non-polio EV pilot surveillance system and to provide both a clinical description of cases and a genetic characterization of viruses, in order to alert other EV reference laboratories and to improve future surveillance and control measures.

3. Study design

An enhanced non-polio EV pilot surveillance system was set up by the Statens Serum Institut to include respiratory specimens obtained from microbiological diagnostic laboratories at Danish hospitals. The new enhanced surveillance of respiratory samples is based upon the existing WHO poliovirus surveillance where stool from meningitis patients with EV-positive cerebrospinal fluid is further tested and characterized with regards to EV subtype and strain specificity [11]. For the enhanced respiratory surveillance, only two of the major hospitals have been included in the pilot test phase. The aim is to test and evaluate the usefulness and added value of the enhanced system before implementing respiratory surveillance full scale. After confirmation of EV diagnostic [12], all EV positive samples received through the enhanced non-polio EV pilot surveillance system were genotyped by sequencing fragments of the viral capsid protein (VP1, VP2 and VP4 sequences) [13,14]. New primers were also designed to sequence a larger VP1 fragment (C104VP1F 5-ATGACTTCTCAGAAGGAGGGTACAT-3′, C104VP1R 5′-TAATCAACTGATCGCCCGACATA-3′, C105VP1F 5′-TACCAAACAGCCATAGTRGTYCCT-3′, C105VP1R 5′-CACCACACTCTCACATGCTT-3′, C109VP1F 5′-GAGGGTGGCTACATAACAGCCTTCT-3′, C109VP1R 5′-GACACCACACCCTTATGTGCT-3′. The VP1 region of EV-D68 positive samples was sequenced using a previously published assay [15]. PCR products were purified using exo-SAP IT (GE Healthcare, Buckinghamshire, UK) prior to Sanger sequencing. Sequences were assembled in BioNumerics v6.6 (Applied Maths, Belgium) and Geneious. EV-C104, C105 and C109 sequences were aligned against all available EV-C104, C105 and C109 sequences from GenBank, as well as a sequence of EV-C116, C117 and EV-D68 using MAFFT [16]. EV-D68 sequences were aligned against other EV-D68 sequences from the B3 lineage as well as EV-D68 sequences from samples collected in Denmark in 2014. Sequences were annotated in SSE [17]. Phylogenetic trees were constructed in MEGA6 [18] using the maximum likelihood method (Kimura 2-parameter model, gamma distribution, invariable sites). Bootstrap analysis was performed using 1000 replicates. EV-C104, C105, C109 and D68 sequences were deposited in GenBank (accession numbers KX901637-KX901644, KY457569-KY457572). Clinical information was obtained for each case. Informed consent from patients was not required according to Danish legislation regarding use of samples collected for surveillance purposes.

4. Results

Between January 2015 and October 2016, a total of 673 EV positive samples were received. Of those, 221 were respiratory samples. Species typing was successful for 70% of the respiratory samples. The following species were identified: EVA in 57 samples, EVB in 27 samples, EVC in 7 samples and EVD in 9 samples. Rhinovirus (RH) A was found in 33 samples, RHB in 6 samples and RHC in 17 samples. EV-C104, C105 and C109 were identified in two, one and three EVC positive respiratory samples, respectively (Table 1). Stool samples were not available for

Table 1
Enterovirus C104, C105, C109 and D68 cases in Denmark, January 2015–October 2016.

Case	Age (years)	Sex	Clinical information	Underlying disease	Location	Sampling date	Respiratory sample material	Diagnostic findings	Phylogeny		
									VP1	VP2	VP4
1	4	F	–	–	Fynen	January 2015	Throat swab	EV C104	–	Yes	–
2	1	M	–	–	Lolland	October 2015	Unknown	EV C105	Yes	–	–
3	4	F	ILI[a]	Immuno-compromised	Zealand	November 2015	Nasal swab	EV C109	Yes	–	Yes
4	24	F	ILI[a]	None	Zealand	January 2016	Unknown	EV C104	Yes	Yes	Yes
5	14	M	ILI[a]	Immuno-compromised	Zealand	January 2016	Unknown	EV C109	–	–	–
6	6	F	ILI[a]	Immuno-compromised	Zealand	March 2016	Nasal/throat swab	EV C109	–	–	Yes
7	18	M	Headache, fever, vesicular rash, diarrhoea, vomiting, respiratory symptoms, chest pains	–	Jutland	August 2016	Expectoration	EV-D68	Yes	–	–
8	1	M	ILI[a]	–	Jutland	August 2016	NP secretion[b]	EV-D68	Yes	–	–
9	14	F	Pneumonia	None	Fynen	September 2016	Unknown	EV-D68	Yes	–	–
10	1	M	–	–	Jutland	September 2016	NP secretion[b]	EV-D68	Yes	–	–
11	4	F	–	–	Zealand	September 2016	BAL[c]	EV-D68	–	–	–
12	6	F	–	–	Zealand	September 2016	NP secretion[b]	EV-D68	–	–	–
13	4	F	–	–	Zealand	September 2016	NP secretion[b]	EV-D68	–	–	–
14	1	F	–	–	Zealand	September 2016	NP secretion[b]	EV-D68	–	–	–
15	0.6	M	–	–	Zealand	September 2016	Tracheal secretion	EV-D68	–	–	–

[a] ILI: influenza-like illness.
[b] NP: nasopharyngeal.
[c] BAL: bronchoalveolar lavage.