Isolating Asian enterovirus 71 subgenogroup C4 in two Austrian clinical samples from 2004

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Hamaguchi *et al.* recently reported on the on the occurrence of enterovirus type 71 (EV71) subgenogroup C4 in Japan [1]. According to the authors, this strain may have emerged in mainland China and in Taiwan. EV71 subgenogroup C4 has recently gained public health interest following reports of an ongoing outbreak in China and Vietnam in 2008: In June 2008, more than 176,000 cases of hand, foot and mouth disease (HFMD) were reported in China alone, and at least 24 deaths have been attributed to EV71 [2,3]. In the largest and most severe EV71-associated HFMD outbreak in Taiwan in 1998, 405 children had severe neurological complications and/or pulmonary oedema; 78 children died [4].

The genus enterovirus comprises poliovirus, coxsackievirus, echovirus, and enteroviruses 68-71. Based on molecular classification, human enteroviruses (HEV) are divided into groups A-D. There are now in excess of 100 types of EV. EV71 has been allocated to the group HEV-A (together with coxsackievirus A16 and some other coxsackie A-viruses) [5]. Both EV71 and coxsackievirus A16 virus can cause HFMD and herpangina, but only EV71 frequently leads to severe diseases, such as aseptic meningitis or poliomyelitis-like paralysis; fatal cases due to pulmonary oedema have been reported in neonates [6].

By molecular typing EV71 can be divided into three genogroups (A,B,C), the latter two being further sub-divided into B1-5 and C1-5 [7,8]. Currently genogroups B and C are co-circulating worldwide. Genotype C1 is predominating in Europe, but it can also be found in Australia, Malaysia and Singapore [9,10]. In China, Taiwan and Japan, the other genotypes are dominant, obviously replacing each other in circulation every one or two years [9,10].

Laboratory investigation in Austria

In Austria, the Agency for Health and Food Safety (AGES) serves as the national reference centre for laboratory diagnosis of poliomyelitis [11]. Between 1999 and 2007, 1,388 stool specimens from patients with acute flaccid paralysis (AFP) or aseptic meningitis were voluntarily submitted by hospitals, none of them yielding polio virus.

The number of reported AFP-cases in patients under 15 years-old per year was 12 in 1999 (0.87/100,000/year), 13 in 2000 (0.95/100,000/year), nine in 2001 (0.66/100,000/year), eight in 2002 (0.62/100,000/year), two in 2003 (0.16/100,000/year), seven in 2004 (0.53/100,000/year), three in 2005 (0.23/100,000/year), and nine in 2006 (0.69/100,000/year) [11]. The WHO requirement of testing two stool specimens (gained within two weeks after onset of paralysis) for enterovirus in at least 80% of AFP-cases was fulfilled in 2002 (88%) and in 2003 (100%).

Stool specimens were processed according to recommendations from the World Health Organization (WHO) listed in the WHO's 'Polio laboratory manual' [12]. In brief, samples were treated with chloroform and antibiotics to remove bacteria and fungi. Cell cultures were inoculated, incubated at 36°C and observed for cytopathic effect (CPE) daily. When complete CPE was obtained, the infected cells were harvested and stored at -20°C until serological typing by neutralisation tests using a kit provided by the National Institute of Public Health and the Environment (RIVM), Bilthoven, the Netherlands.

Of the 1,388 stool samples, 201 yielded non-polio enteroviruses. A total of 181 viruses were available for molecular typing as

Table

Patients with aseptic meningitis due to enterovirus 71 infection, Austria, 1999-2007

No.	Age in years	Sex	Province	Month and year of sampling	Subgenogroup
1	8	M	Vienna	7/2001	C1
2	6	M	Vienna	7/2001	C1
3	4	F	Styria	3/2002	C1
4	7	M	Styria	7/2002	C1
5	7	M	Styria	8/2002	C1
6	3	F	Styria	10/2002	C1
7	8	M	Styria	10/2002	C1
8	8	F	Styria	11/2002	C1
9	5	F	Styria	4/2003	C1
10	2	M	Styria	7/2003	C1
11	6	M	Styria	7/2003	C1
12	7	M	Styria	7/2002	C1
13	2	F	Styria	7/2003	C1
14	6	F	Styria	8/2003	C1
15	<1*	F	Lower Austria	10/2004	C4
16	8	M	Lower Austria	10/2004	C4

m = male; f = female
* 8 months

described by Nix *et al.* [13]. For EV71-positive isolates, extended length VP1 gene sequences were amplified as described by Oberste *et al.* [14].

Retrospective analysis of enterovirus samples

On the basis of a general cooperation agreement signed between AGES and the Center for Disease Control (CDC) in Taipei in fall 2007, AGES decided to screen retrospectively Austrian enterovirus isolates collected in its acute flaccid paralysis surveillance programme for EV71. Molecular typing was performed by an AGES employee in February 2008 during a three week stay at the Viral Enteric and Emerging Diseases Laboratory at the CDC in Taipei, Taiwan. Analysing enteroviruses cultured from 181 clinical samples collected between 1999 and 2007, we detected EV71 in specimens from 16 patients (8.8%): EV71 genotype C1 was found in 14 cases, and genotype C4 in two cases.

In the Austrian province of Lower Austria (1.5 million inhabitants), an eight year-old boy of Vietnamese descent (born and raised in Austria) and an eight month-old female breastfed infant, were hospitalised in October 2004 at different institutions for respective aseptic meningitis and aseptic meningitis plus diarrhoea. The two children lived in villages approximately 270 km apart, without any known common contacts; they were not known to have traveled abroad.

Table 1 summarises demographic data of the 16 patients with EV71 infection. No signs of HFMD were documented in any of them. All 16 Austrian patients with EV71 infection recovered completely.

Discussion and conclusions

The discovery of two EV71 subgenogroup C4 isolates in Austria was an unexpected finding, as was the obvious occurrence of a cluster of infection with EV71 subgenogroup C1 in the years 2001-2003 in the province of Styria (1.2 million inhabitants). The fact that one of the two Austrian children suffering from EV71 subgenogroup C4 infection was of Vietnamese descent suggests that independent introduction of Asian strains may occur in Europe.

Several EV71 outbreaks have been documented throughout the world and clinical manifestations of EV71 infections can be diverse, including HFMD, herpangina, central nervous system (CNS) disease, and pulmonary oedema [1]. In children, the CNS diseases associated with EV71 manifest clinically in various ways, such as aseptic meningitis or acute flaccid paralysis, symptoms previously well known for infections with poliovirus. After the eradication of wild poliovirus from most parts of the world, EV 71 must be regarded as one of the most dangerous neurotropic enteroviruses. The well established WHO PolioLabNet may be well advised to upgrade for surveillance of EV71. Enterovirus surveillance is already an integral activity in some European polio laboratories.

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