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Stool screening of Syrian refugees and asylum seekers in Germany, 2013/2014: Identification of Sabin like polioviruses

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ABSTRACT

Germany is a partner of the Global Polio Eradication Initiative. Assurance of polio free status is based on enterovirus surveillance, which focuses on patients with signs of acute flaccid paralysis or aseptic meningitis/encephalitis, representing the key symptoms of poliovirus infection. In response to the wild poliovirus outbreak in Syria 2013 and high number of refugees coming from Syria to Germany, stool samples from 629 Syrian refugees/asylum seekers aged <3 years were screened for wild poliovirus between November 2013 and April 2014. Ninety-three samples (14.8%) were positive in an enterovirus specific PCR. Of these, 12 contained Sabin-like polioviruses. The remaining 81 samples were characterized as non-polio enteroviruses representing several members of groups A–C as well as rhinovirus. Wild-type poliovirus was not detected via stool screening involving molecular and virological methods, indicating a very low risk for the importation by Syrian refugees and asylum seekers at that time.

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1. Introduction

Human enteroviruses (EV) belong to the *Picornaviridae* family and constitute a large genus of viruses containing more than 110 different types that can be classified into four species (EV-A, -B, -C, and -D). Three species of rhinoviruses have also been included recently into this genus (Knowles et al., 2012). EV are small non-segmented viruses with a positive-strand RNA genome of about 7500 kb, surrounded by an icosahedral capsid composed of VP1–VP4 proteins (Wimmer et al., 1993).

Enteroviruses can cause a wide spectrum of acute diseases, ranging from mild fever and respiratory illness to severe neurologic diseases with involvement of central nervous system such as aseptic meningitis and poliomyelitis (Melnick, 1983). The latter is a severe, but vaccine-preventable disease that is caused by polioviruses (PV) types 1–3 which are members of EV-C species (Minor, 2014).

Only 0.1–1% of polio infections result in acute flaccid paralysis (AFP) of the limbs. Person-to-person spread of PV via the faecal–oral route is the most important way of transmission, although the oral–oral route may account for some cases. The virus can be shed in the stool from three to six weeks (Melnick, 1996). Persons with primary immune deficiencies can excrete the virus for several months and even years (Burns et al., 2014), leading to accumulation of mutations characteristic for wild type virus genotype.

Worldwide concerted efforts under the leadership of the World Health Organization (WHO) and its partners have been successful in reducing the incidence of poliomyelitis which is now targeted for global eradication by the year 2018 (Dowdle and Birmingham, 1997; Global Polio Eradication Initiative, 2014). The transmission of wild-type polioviruses (WPV) has been interrupted in four of the six WHO regions and now more than 80% of the world's population lives in certified polio-free regions. There are only three countries with endemic WPV circulation left, namely Pakistan, Afghanistan and Nigeria (http://www.polioeradication.org/).

As humans are the only reservoir for PV, travel between polio-free countries and those where WPV circulates will largely determine the risk of the virus being re-imported.

Although the WHO European region was officially declared polio-free in 2002, importations of WPV have occurred and resulted in an outbreak as recently as 2010 in Tajikistan (Yakovenko et al.,

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2014) or a silent circulation with detection in sewage in 2013–2014 in Israel (Manor et al., 2014).

The lowest number of global polio cases ever reported was in 2012 with 223 confirmed cases. In 2013, this number increased to 416. To protect the overall global progress, WHO declared WPV spread as a Public Health Emergency of International Concern under the International Health Regulations. The rationale was the observation that in 2013 about 60% of polio cases were the result of international spread of WPV. Even during the low-transmission season for polio, the virus has been exported to three countries in three major epidemiological zones: in central Asia (from Pakistan to Afghanistan), in Central Africa (Cameroon to Equatorial Guinea) and in the Middle East (Syria to Iraq).

After being polio-free since 1999, a polio outbreak with 36 confirmed cases occurred in Syria from October 2013 to January 2014. WPV type 1 originating from Pakistan was found in all stool samples investigated (Aylward and Alwan, 2014). Because of the civil war in Syria, the vaccination has been discontinued since 2011 and the officially reported polio vaccination coverage decreased from 99% in 2010 to 68% in 2012 (http://www.who.int/immunization/monitoring_surveillance/data/syr.pdf). This puts children born after 2010 at the highest risk of acquiring polio or transmitting WPV.

In 2012, Syrians became the single largest group of persons granted protection status in the European Union. More than 70% were recorded in only two Member States: Germany and Sweden. In 2013, 11.851 asylum applicants from Syria were registered in Germany with increasing number in 2014 (http://www.bamf.de/SharedDocs/Anlagen/DE/Publikationen/Broschueren/bundesamt-in-zahlen-2013.pdf?__blob=publicationFile).

Due to the WPV outbreak in Syria and high number of refugees coming from Syria, Robert Koch Institute (RKI) as the national public health institute in Germany re-emphasized the necessity of polio vaccination using inactivated polio vaccine (IPV) for all residents and staff of asylum seeker reception centres and shelters. Additionally, after consultation with the National Certification Committee (NCC), mandatory testing of stool samples of asymptomatic Syrian refugees and asylum seekers aged less than three years was recommended. The aim of this study was to evaluate the absence of WPV. Timely identification of poliovirus transmission is critical to the control of polio. Every single clinical case of polio in a polio-free area must be addressed to prevent further cases.

The screening of stool samples for EV/PV as well as characterization of EV-positive samples and the intratypic differentiation (ITD) of all PV was performed by the National/WHO Regional Reference Laboratory for Poliomyelitis and Enteroviruses (NL/RRL) at RKI. In addition, three labs of the German EV-laboratory network also performed primary testing of stool samples using molecular assays as well as cultivation on different cell lines. The German EV-network was established for continuous monitoring the polio-free status of Germany, performed by investigation of stool samples from patients with viral meningitis or encephalitis as well as AFP.

2. Materials and methods

2.1. Samples

During a period of six months (November 2013–April 2014) 629 stool samples from Syrian refugees and asylum seekers were screened for EV by molecular and virological methods. Samples tested positive in the EV-network labs (Hanover, Stuttgart, Oberschleissheim) were sent to the NL/RRL for further differentiation. Overall, 414 samples were analyzed at the NL/RRL. Data requested included name, date of birth, date of arrival in Germany, travel history, polio vaccination history and health status. For final analysis, personal data were handled pseudonymously.

2.2. Sample processing and RNA extraction

Stool samples sent to the four labs were processed according to the individual protocol. Samples analyzed at the NL/RRL were prepared according to the WHO-Polio Laboratory Manual (WHO manual, 2004). Faecal samples were treated with chloroform, centrifuged and supernatant was recovered and used for RNA isolation and cell culture. RNA was extracted from 140 μl stool suspension including 10^3 pfu of MS2 phage as internal control with QIAmp Viral RNA mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. RNA was subsequently used for screening PCR and stored at −20 °C until further use.

2.3. Enterovirus detection and molecular typing

Screening for EV in the four labs was done by using three different PCR systems targeting 5′ non-coding region (5′NCR) (Hyypiä et al., 1989; Kuryk et al., 2014, Argene/Bio-Merieux Enterovirus Kit). At the NL/RRL screening for EV RNA was carried out using primers published recently (Kuryk et al., 2014). Internal control was detected in a RT realtime PCR setting. Internal control RT-PCR with Cp values +3 compared to the value of negative control set as reference were supposed to be inhibited and 5′NCR screening PCR was repeated with RNA diluted 1:5 and 1:10.

For molecular typing different PCR systems were used: locked nucleic acid (LNA) modified CODEHOP primers designed for amplifying partial VP1 region of all enterovirus serotypes (Nix et al., 2006) and EV group A and B specific PCR assays for increased sensitivity (Leitch et al., 2009, 2011; Diedrich et al., 2009).

2.4. Sequencing and sequence analyses

Sequencing was done directly on amplification product using BigDye 3.1 (Applied Bio systems). Two different sequence analysing tools were used: BLAST (Altschul et al., 1990) and EV typing tool (Kroneman et al., 2011).

2.5. Cells and virus isolation

In accordance to WHO guidelines for PV isolation, RD-A and L20B cell lines were used for all samples analyzed at the NL/RRL. Additionally, PCR positive samples were also inoculated on CaCo-2 and HEp-2C cells.

Inoculation of stools samples was done into cell suspension; time schedule for passaging on RD-A, L20B and CaCo-2 cells was seven days and five days for HEp-2C cells. Up to three passages were carried out.

2.6. Virus neutralization test

Isolate characterization was performed by microneutralization test using type specific neutralizing antibodies (RIVM pool sera and in-house monospecific sera) based on the VP1 sequencing results. If cytopathic effect (CPE) developed under immune sera, inoculum was harvested and passaged. VP1 sequence from resulting isolate was compared to original sequences and in case an additional virus type could be identified, neutralization test was repeated using both sera.

2.7. Intratypic differentiation (ITD) of Polioviruses

According to WHO-guidelines, ITD between wild and Sabin (vaccine)-like PV was done by PCR (WHO manual, 2004). In

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