Detection of non-polio and polio enteroviruses in Acute Flaccid Paralysis surveillance in Turkey

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ABSTRACT
Poliomyelitis was a disease feared worldwide, striking suddenly and paralysing mainly children for life. Monitoring of suspected cases of poliomyelitis is carried out with Acute Flaccid Paralysis (AFP) surveillance in Turkey. This study examines national data of AFP surveillance and the epidemiology of enteroviruses (EV) in Turkey from 2000 to 2019 and gives an overview of the detected serotypes of EVs. A total of 13,640 samples collected from patients with 5216 AFP pre-diagnosed cases (2 samples from each patient) and 3,208 contacts, during a 20-year period (2000–2019) were investigated. All isolated polioviruses were tested for their wild or vaccine origin according to the WHO recommended protocol by PCR and sequencing analysis were performed. Enterovirus positivity was detected in a total of 915 cases, which were identified as 204 Sabin-like polio virus (SLPV) and 711 non-polio enterovirus (NPEV). Of the 204 SLPV, 141 (69.1%) AFP were detected in patients and 63 (30.9%) were detected in samples taken from their contacts. Of the 711 NPEVs, 516 (72.5%) were from AFP cases and 195 (27.5%) were detected in samples taken from their contacts. It is concluded that the reason for the higher detection rate of NPEV in samples from AFP pre-diagnosed cases is attributed to the polio vaccination rates reaching 97% between 2008 and 2019 in Turkey. The most frequently detected NPEV serotypes were Coxackie A24, B3, and Echo 30. This retrospective study is the first comprehensive study in Turkey to evaluate the results of the AFP surveillance in the last 20 years.

KEYWORDS
enteroviruses, polio virus, surveillance, Turkey

INTRODUCTION
Human enteroviruses (HEV) are non-enveloped, single-stranded, positive-sense RNA viruses belonging to the Picornaviridae family and Polioviruses the causative agent of polio are a member of enteroviruses. Up to now, 15 types have been identified. Human enterovirus serotypes have traditionally been classified into Polioviruses (serotype 1–3), Coxsackievirus A (serotype 1–22, 24), Coxsackievirus B (serotype 1–6) and Echovirus (serotype 1–33) [1]. Enteroviruses are among the most common viral agents of infection in humans. Although HEV often cause asymptomatic or mild infections, they also cause severe illnesses such as encephalitis, meningitis, aseptic meningitis, hand-foot-and-mouth disease, acute haemorrhagic conjunctivitis, Acute Flaccid Paralysis (AFP), herpangina, myalgia and myocarditis.
Materials and Methods

Two samples of stool are being obtained from all AFP cases in first 2 weeks of their illness and are sent to laboratory for virus isolation.

Clinical samples

Obtaining stool samples. Stool samples were collected twice within a 24–48 hour intervals from AFP-suspected cases sent to the laboratory with a case investigation form by the health care workers in provincial health directorates. Patients in risk groups such as having a history of less than 3 vaccine doses, travelling to a polio endemic country, and unknown vaccination status were considered hot cases. Stool samples were collected from five children under five years of age who had close contact with these cases. A total of 13,640 samples collected from patients with 5216 AFP pre-diagnosed cases (two samples from each patient) and 3,208 contacts, during a 20-year period (2000–2019) were investigated. Stool samples were sent to our laboratory via a cold-chain transferring system. All cases were followed for 60 days to identify probable residual paralysis cases. All stool samples were processed with the recommended WHO algorithm for enteroviruses [10]. Information about the patient such as symptoms, onset date of symptoms, vaccination status, travel history, sample collection date, name of contacts etc. were recorded on the investigation form.

Processing stool samples. Labelled centrifuge tubes with sample numbers and added 10 ml phosphate buffer solution (PBS), 1 g of glass beads and 1 ml chloroform to each tube. Transferred approximately 2 g of each faecal sample in the biological safety cabinet (BSC), and shaken vigorously for 20 minutes using a mechanical shaker. Finally spin for 20 minutes at 1,500 g in a refrigerated centrifuge. Working in a BSC, transferred supernate from each sample into two labelled storage vials [10].

Virus isolation and identification

All samples were inoculated onto human rhabdomyosarcoma cells (RD), and mouse cell lines (L20B) recommended by WHO for isolation of PV and other HEVs. Cell cultures exhibiting a cytopathic effect (CPE) were serotyped with antisera pools from RIVM Institute (Bilthoven-Netherlands) until August 2015 for differentiation of PV and NPEV. Neutralisation assays has been performed according to WHO recommendations [10]. After 2015 molecular techniques has been set up into the laboratory and algorithm for

Enteroviruses (EVs) are widespread globally. In the northern hemisphere, they often cause infection in May-October except rhinoviruses, and in tropical regions, they cause infection year-round. Although they are mainly transmitted by the faecal-oral route, they may also spread via droplet and may cause respiratory infection. Improper hygienic conditions and crowded life conditions increase the spread of the virus. Contamination of water via sewage systems causes epidemics [2–4]. Poliovirus is the most significant member of the EV in terms of public health, causing AFP. Poliovirus isolates are grouped into three categories: Wild poliovirus (WPV), vaccine-related poliovirus, and vaccine-derived poliovirus (VDPV). WPV has 3 serotypes, classified as Type-1, Type-2 and Type-3. Vaccine-related polioviruses have limited divergence in the capsid protein (VP1) nucleotide sequences from the corresponding OPV strain (poliovirus type 1 and 3 [PV1 and PV3]; ≤1 % divergent; poliovirus type 2: ≤0.6 % divergent). VDPVs are >1 % divergent (for PV1 and PV3) or >0.6 % divergent (for PV2) in VP1 nucleotide sequences from the corresponding OPV strain [4]. VDPV’s are further classified as 1) cVDPVs, when evidence of person-to-person transmission in the community exists; 2) iVDPVs, when they are isolated from persons with primary immunodeficiency diseases (PIDs); and 3) aVDPVs, when they are clinical isolates from persons with no known immunodeficiency and no evidence of transmission, or they are sewage isolates that are unrelated to other known VDPVs and whose source is unknown [5]. Two types of vaccine have been used for immunization of poliovirus, oral poliovirus vaccine (OPV) and inactivated poliovirus vaccine (IPV). OPV viruses have to ability to spread to, infect and immunize unvaccinated contacts of vaccine recipients, as immunologically normal individuals usually excrete infectious vaccine viruses for 2–4 weeks after receiving OPV [6, 7]. Despite its well-established safety record, OPV use can be associated with rare emergence of genetically divergent vaccine-derived polioviruses (VDPVs) whose genetic drift from the parental OPV strains indicates prolonged replication or circulation [5]. In addition, circulating vaccine-derived polioviruses (cVDPVs) can emerge very rarely among immunologically normal vaccine recipients and their contacts in areas with inadequate OPV coverage and can cause outbreaks of paralytic polio. Cessation of all OPV use after certification of polio eradication will eliminate the risk for new VDPV infections. The Polio Eradication Programme (PEP) was initiated in 1989 by the World Health Organization (WHO), for monitoring and eradicating polio virus globally. In order to reach this aim, PEP was initiated in Turkey in 1989 [8]. The polio vaccine started to be used in our country in 1963. After the implementation of the polio eradication program in 1989, the national polio vaccination days began in 1995 [9]. Clinical classification was used for AFP cases between 1989–1997. After 1997, virological classification began to be carried out. In addition to OPV vaccination, IPV vaccine was added to the vaccine schedule in 2010 under this program [8]. The last wild poliovirus had been isolated in Turkey on 26 November 1998. In the following years, the European region, including our country, was designated as a polio-free region at the headquarters of WHO on 21st June 2002. This paper reports the results of AFP surveillance in Turkey from 2000 to 2019 and gives an overview of the detected serotypes of enteroviruses and their patterns of occurrence in polio-suspected paralytic cases and healthy contacts.
identification of isolates has been changed to the real time based PCR assays as Intratypic Differentiation (ITD) method developed by CDC (Atlanta, USA). All isolated polioviruses were tested for their wild or vaccine origin according to the WHO recommended protocol by PCR and sequencing analysis were performed [11].

RESULTS

A total of 13,640 stool samples of 8,424 cases monitored under AFP surveillance for 20 years were analysed. 5,216 of 8,424 cases belonged to AFP cases, 3,208 of them were contacts. EV positivity was detected in a total of 915 cases, which were identified as 204 Sabin-like polio virus (SLPV) and 711 NPEV. A total of 204 samples were SLPV positive, including 141 (69.1%) AFP cases and 63 (30.9%) contacts of the cases. A total of 711 samples were NPEV positive including 516 (72.5%) AFP cases and 195 (27.5%) contacts (Figs. 1 and 2).

The distribution of SLPV serotypes detected in the AFP cases and their contacts, between 2000 and 2019, is shown in Fig. 3 out of 711 NPEV-positive samples, 99 (13.9%) were CoxA serotypes, 65 (9.1%) were CoxB serotypes, 170 (24.0%) were Echovirus (EchoV) serotypes and 15 (2.1%) samples were other EV serotypes. 362 (50.9%) EV-positive samples were not serotyped and were reported as NPEV (Fig. 4). The most frequently detected EV serotypes were CoxA24 (7.42%), CoxB3 (4%) and Echo 30 (3.23%). All other serotypes were detected in cases received within the scope of AFP surveillance. Although NPEVs are seen in every age group, the detection rate in the 1–4 year age group

![Fig. 1. Distribution of SLPV/NPEV in AFP cases according to the years](image1)

![Fig. 2. Distribution of SLPV/NPEV in contact cases according to the years](image2)
was mere common 44.5% (Fig. 5). The detection rate was 1.6 times higher in males than females. Since 1998, wild poliovirus has not been detected in Turkey, only vaccine strains, SLPV were detected. The incidence of AFP cases increased from 1.03 cases/100,000 individuals in 2000 to 1.66 cases/100,000 individuals in 2019 (Fig. 6). The polio vaccination rate reached from 80% between 2000–2008 to 97% between 2008–2018. Organizing national vaccination days and vaccination campaigns covering all children under the age of 5 contributed greatly to the increase in these rates (Fig. 7).

DISCUSSION

Until poliomyelitis is totally eradicated globally, there is always a risk of importation of wild poliovirus to Turkey. In the event of an imported poliomyelitis case, with early detection and proper timely interventions, the spread of the virus in the country may be prevented. Unless proved otherwise by the laboratory, all AFP cases must be considered as polio. This definition encompasses paralysis due to PV or NPEV, polyneuropathies such as Guillain-Barre Syndrome/Landry syndrome, transverse myelitis, traumatic neuropathies excluding severe traumas, cord tumours (acute cord injury, haematoma, abscess, etc.) or neoplasms, peripheral neuropathies due to infection (diphtheria, borrelia, etc.) or intoxications (tick paralysis, snakebite, heavy metal poisoning, poisoning with insecticides, etc.), specific neurologic diseases, systemic or metabolic diseases, and skeletal or muscular diseases [12]. In addition, it also encompasses acute paralyses, whose AFP aetiologies are not known or defined. In AFP surveillance studies carried out as part of PEP, to prove that polio virus-positive cases are not due to wild polio virus is necessary. At least one case of non-polio AFP should be detected annually per 100,000 population aged less than 15 years to prove that AFP surveillance programme is effective. This criterion is an indicator of the functioning of the polio surveillance system [13].
incidence, which was 1.03/100,000 in 2000, reached 1.35/100,000 in 2019 as a result of strengthening the quality of AFP surveillance in Turkey. AFP and permanent paralysis have been reported even in regions where poliovirus has been eradicated in the world. In a study conducted in Nigeria between 2002–2003, it was reported that 14.6% of AFP cases were due to NPEV and that it caused permanent paralysis in 24 cases where Echoviruses were isolated [14].

Shahmahmoodi et al. 2008; reported that although poliovirus was eradicated in Iran in 2001, the number of cases

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**Fig. 5.** Distribution of the most common NPEV according to age groups

**Fig. 6.** AFP incidence of Turkey, 1990–2019

**Fig. 7.** Polio cases and the comprehensiveness of Polio-3 (DPT+IPV+Hib 5-in-1 vaccine, 3rd dose), Turkey, 1985–2018.

PEP: Polio Eradication Program; NVD: National Vaccination Days; MOP-UP: Mopping-up
rose from 400 in 2004 to 600 in 2007 [15]. Over 16 years, 52 different NPEV serotypes were detected in Turkey. The most frequently detected EV serotypes were Cox A24, Echo 30 and Cox B3. Other frequently isolated serotypes were CA4, CA9, CB4, CB5, E14 and E03. During the period of study, some serotypes were isolated only once or twice. These serotypes were considered to be sporadic cases. In our study, when the distribution of NPEV agents according to their ages are examined, it is seen that the most common cases are between 1 and 4 years old, and Cox A24 is most frequently seen. Although enteroviruses can infect any age group, they cause infections in small children more frequently [16, 17]. The frequency of NPEV detection differs throughout the year. In many studies conducted, it was shown that Coxsackievirus B3 was not only related to acute haemorrhagic conjunctivitis, but was also frequently isolated from AFP cases [18–20]. While Coxsackievirus B3 caused diseases such as myopericarditis, aseptic meningitis, meningoencephalitis, herpangina and rash, it was also isolated from AFP cases [21–24]. Enterovirus 71 causes various clinical presentations and terminal diseases (such as aseptic meningitis, respiratory tract infections, myocardiatis, paraletic diseases and hand-foot-and-mouth disease). Bahri et al. 2005; detected Echo virus 6, 11 and 30 serotypes most frequently in an EV surveillance study based on 12-year AFP surveillance that they conducted in Tunisia [18]. As eradication activities towards poliovirus advance globally, non-polio enteroviruses (NPEVs) will gain more importance as a factor in AFP. In addition, in our study, the OPV vaccine strain was the second most frequently identified factor in clinical samples receiving an AFP pre-diagnosis. We believe that as long as AFP continues to be included in OPV vaccination programmes, we will frequently observe AFP in the laboratory diagnosis. After 2000, wild poliovirus and vaccine-derived viruses were detected in countries bordering Turkey (Georgia, Syria, and Iraq, others). Vaccination support activities designed to “mop-up” polio cases have been organized in some provinces of Turkey to strengthen the vaccination rates in border region. The ratio of vaccine strains detected in AFP cases has increased since 2000 due to the use of the oral polio vaccine (OPV). When refugees were adopted from Syria in 2012, additional vaccination activities were conducted. Therefore, in 2013 and 2014, the ratio of SLPV strains detected in AFP cases increased. At the start of the PEP, only OPV was used in Turkey. The main purpose of AFP surveillance is the early diagnosis of local or imported poliomyelitis cases, infection with wild poliovirus, and taking any required measures.

REFERENCES


