Summary report on the Nineteenth intercountry meeting of directors of poliovirus laboratories in the Eastern Mediterranean Region

Tunis, Tunisia
23–25 October 2017
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1. Introduction

The nineteenth intercountry meeting of directors of national and regional poliovirus laboratories in the Eastern Mediterranean Region was held in Tunis, Tunisia, on 23–25 October 2017. The meeting was attended by directors of poliovirus laboratories in 12 countries of the Region, members of the Eastern Mediterranean Regional Certification Commission and national certification committees, and technical experts from: Centers for Disease Control and Prevention (CDC), United States of America; Kenya Medical Research Institute (KEMRI), Kenya; National Institute for Biological Standards and Control (NIBSC), United Kingdom; National Institute for Public Health and the Environment (RIVM), Netherlands; National Institute for Health and Welfare, Finland; and the National Polio Laboratory, Turkey. WHO staff from headquarters, country offices and the Regional Office for the Eastern Mediterranean also attended.

The objectives of the meeting were to:

- review the poliovirus regional laboratory network performance;
- provide technical information on issues related to the global polio eradication programme;
- discuss the role of polio laboratories in polio endgame strategy Global Action Plan III (GAPIII) Phase 1 activities and environmental surveillance; and
- develop recommendations for further improvement in laboratory performance.

Dr Yves Souteyrand, WHO Representative to Tunisia, delivered a message on behalf of Dr Jaouad Mahjour, acting Regional Director for the Eastern Mediterranean, noting the high level of commitment and progress made towards the eradication of poliomyelitis in the Region. He commended countries for maintaining high surveillance standards
and highlighted the strategies adopted to eradicate wild poliovirus transmission in the two remaining endemic countries, Afghanistan and Pakistan, as well as the actions taken to supplement acute flaccid paralysis (AFP) surveillance with environmental surveillance in at-risk countries and to maintain the highly efficient environmental surveillance in Afghanistan, Egypt and Pakistan, and the progress made on GAPIII Phase 1a and 1b activities for the containment of polioviruses and potential infectious material.

The first session of the meeting provided a regional overview of the progress on polio eradication activities in the Eastern Mediterranean Region during 2016. Surveillance standards in countries have been maintained with significant improvement in diagnostic capacities through the introduction of poliovirus intratypic differentiation (ITD) at national poliovirus laboratories in Iraq, Jordan, Sudan and the Syrian Arab Republic. The recently established environmental surveillance laboratory in Jordan is now fully functional and serves as a reference laboratory for testing environmental samples collected in Lebanon. The well-organized network of poliovirus laboratories in the Region has facilitated the establishment of a genomic sequencing facility at the regional reference laboratory in Egypt with the support of the regional reference laboratory for polio eradication in Pakistan. Technical cooperation such as this enables countries to strengthen their diagnostic capacities and services.

2. Summary of discussions

Laboratory network performance and challenges dealing with the workload

All polioivirus laboratories in the Eastern Mediterranean Region Polio Laboratory Network (EPLN) are fully accredited. The quality of laboratory performance indicators in the EPLN, including for Iraq and
Syrian Arab Republic, which are working under challenging security conditions, has been maintained to certification standard. However, the high workload of some laboratories, such as those in Egypt and Pakistan, hinders their ability to maintain the timeliness and accuracy of results.

The regional reference laboratory in Pakistan also serves as the national laboratory for Afghanistan and tests more than 20,000 stool samples annually from AFP cases and their contacts. The laboratory is also testing stool samples collected from healthy and nomadic children in high-risk areas. Both Afghanistan and Pakistan have expanded environmental surveillance and increased the number of sample collection sites; as of October 2017, there were 53 sites in Pakistan and 20 in Afghanistan. In 2017, Pakistan’s regional reference laboratory established the first regional poliovirus serology laboratory to test 16,000 serum samples collected from three districts in the country, supported by the Bill and Melinda Gates Foundation, CDC and WHO. In addition, the regional reference laboratory is involved in the pilot testing of a new sampling tool, the bag-mediated filtration system for environmental surveillance developed by PATH and the University of Washington, in 10 different cities in Pakistan.

The regional reference laboratory in Egypt supports AFP surveillance in countries without testing facilities, such as Yemen, and provided ITD testing of poliovirus isolates referred from countries, including Iraq, Jordan, Lebanon, Sudan and the Syrian Arab Republic, during the recent serotype-2 vaccine-derived poliovirus (VDPV2) outbreak. Similarly, the KEMRI poliovirus laboratory is supporting the testing of AFP and contact samples collected in Djibouti and Somalia. The regional reference laboratory in Egypt has acquired expertise in poliovirus nucleotide sequencing and is supporting environmental surveillance in the country, testing 44 samples per month collected from all 27 governorates. The Egyptian Ministry of Health and Population is
conducting primary immune deficiency disorders (PID) surveillance to detect any possible excretion of poliovirus in children diagnosed with immune deficiency. From 2011 to 2017, stool samples from 250 PID patients were tested, with the detection of immunodeficiency-related vaccine-derived poliovirus (iVDPV) in 10 patients.

Jordan’s national poliovirus laboratory serves Lebanon and the Syrian Arab Republic by testing stool samples collected from paralytic cases. In November 2016, the laboratory established an environmental surveillance laboratory with three sampling sites covering areas with high population density and considered at greater risk of poliovirus importation due to shared borders with the Syrian Arab Republic. The laboratory is also supporting environmental surveillance testing of sewage water in Lebanon and conducted a WHO training workshop for Iraqi national poliovirus laboratory staff on processing environmental sewage water samples. Staff from Jordan and Iraq’s national poliovirus laboratories have been trained on real-time polymerase chain reaction (PCR) assays used for poliovirus ITD and vaccine-derived poliovirus (VDPV) testing, and are parallel testing L20B positive samples to be able to perform PCR independently, after passing the proficiency testing panel and acquiring accreditation status.

Molecular epidemiology of wild poliovirus and vaccine-derived poliovirus

A detailed picture of contemporary poliovirus strains circulating in was presented. On the basis of genetic information, progress towards polio eradication in the two endemic countries of Eastern Mediterranean Region, Afghanistan and Pakistan, is very encouraging. A sustained decline in genetic diversity with disappearing clusters and lineages is clear, although virus circulation still continues in reservoir areas and poses a risk to areas with low immunity, alongside the
continuous risk of reseeding non-reservoir areas. Outside reservoir areas, population movement in communities with immunity gaps throughout the country presents another risk that needs to be urgently addressed. Continued wild poliovirus (WPV1) transmission was seen in the Hilmand and Nangarhar provinces of Afghanistan during 2016 and 2017. Environmental surveillance in Afghanistan and Pakistan has been proven to be a powerful tool to detect wild poliovirus circulation in the absence of poliomyelitis cases in many communities.

A massive outbreak of circulating vaccine derived poliovirus type 2 (cVDPV2) has been reported in the Syrian Arab Republic. As of 10 October 2017, 48 cVDPV2 isolates have been detected in six districts in three governorates (Mayadeen, Deir Ez-Zor, Boukamal, Tell Abyad, Thawra and Tadmour). A total of 303 AFP cases and 584 contact cases have been tested in collaboration with five Global Polio Laboratory Network (GPLN) laboratories, including CDC, Public Health Institution of Turkey, RIVM, the Syrian national polio laboratory and VACSERA in Egypt.

The laboratory testing of samples during the Syrian outbreak provided an opportunity to review preparedness capacities at the regional level and demonstrated the efficiency and effective coordination of the GPLN system. Initially, long delays between the collection date and receipt in laboratories were experienced due to difficulties in northern Syrian Arab Republic with the collection and transportation of samples, while surveillance and monovalent oral poliovirus vaccine type 2 (mOPV2) response activities were hindered by conflict in many areas. Inactivated polio vaccine (IPV) vaccination campaigns were carried out in countries neighbouring the Syrian Arab Republic, such as Lebanon and Turkey, to contain the geographical extent of cVDPV2 spread.
Maintaining laboratory quality performance

The causes, control and implications of contamination in poliovirus laboratories was discussed. Additional quality assurance requirements in poliovirus laboratories are required post-eradication of WPV2 and the removal of type 2. The cell culture method of poliovirus isolation is still recognized as the gold standard method for the detection of poliovirus in stool and sewage water samples. The two cell lines used for poliovirus isolation are L20B and RD, and high standards of maintenance are required to ensure that laboratories meet the requirements for optimal cell growth, as minor changes in the growth media and incubation temperature alter the cell sensitivity for poliovirus infection. The testing procedures adopted in poliovirus laboratories should be critically reviewed after on-site risk assessment and following guidance provided in GAPIII for poliovirus containment.

Environmental surveillance

Environmental surveillance should be included in national action plans for poliovirus surveillance and should take into consideration the following planning parameters: the timeframe and schedule of sampling; details of sampling sites (location, population sizes and demographics); responsibilities for sampling, instructions for sampling and sample logistics; provision of adequate laboratory space, personnel, equipment and reagents; standardized protocols for sample collection, transportation, processing and virus identification; data management and reporting systems; and training and quality assurance.

In the Region, environmental surveillance for poliovirus is fully functional in Afghanistan, Egypt, Jordan, Lebanon and Pakistan. Training is in progress to expand environmental surveillance capacities in Iraq, Islamic Republic of Iran, Sudan, Syrian Arab
Republic and Yemen, given the high risk of poliovirus importation in these countries due to their geographical location. WHO is also supporting these countries in establishing environmental surveillance systems by providing technical support and resources related to site selection, sampling and laboratory testing procedures.

Environmental surveillance in Afghanistan covers 20 sampling sites in nine provinces. The data indicates that the detection of poliovirus transmission appears earlier in environmental surveillance samples than the appearance of AFP cases in a particular population. The findings complement existing surveillance capacities to ascertain the extent of transmission and circulation of poliovirus in communities, especially in endemic countries. The environmental surveillance system in Pakistan has also been dramatically expanded to 33 large cities with 53 operational sampling sites covering major reservoir areas in all four provinces.

With continued developments in laboratory surveillance systems, it is essential to update laboratory management systems through continuous improvements and quality standards to be defined and implemented across the GPLN. WHO is currently working on the development of an accreditation checklist based on the current requirements of poliovirus laboratory algorithms, internal and external quality controls, and containment guidelines and standards. New developments include introducing annual accreditation of environmental surveillance laboratories based on a checklist developed exclusively for quality indicators and laboratory systems relevant to environmental surveillance for polio eradication. In addition, a proficiency test panel has been prepared with the support of a global specialized polio laboratory in the Netherlands and is currently being evaluated by two environmental surveillance laboratories and will be distributed in 2018 to all countries in the Region performing environmental testing for poliovirus.
To further improve laboratory diagnostic services, WHO is working with global specialized laboratories to introduce technological interventions for rapid, reliable and high-throughput results, thereby decreasing the turn-around time. One example is the utilization of a next generation sequencing (NGS) platform for the genetic analysis of poliovirus genomes. A key advantage is that it replaces the laborious and lengthy procedures currently used to obtain the genetic sequence, which cover partial genome regions compared to the full-length poliovirus genomes obtained for multiple samples within the same reaction and in a shorter timeframe.

The additional benefits of NGS are a low volume of input ribonucleic acid (RNA), no primer bias, identification of individual strains in mixtures and mutation content at each nucleotide site, and no need for ITD real-time reverse transcription-polymerase chain reaction (rRT-PCR). WHO is currently working on a pre-defined study to rationalize uniform NGS algorithms across the polio laboratory network. Substantial progress has been made with the support of GPLN laboratories in Australia, France, India, Japan, Netherlands, UK and USA who are involved in the pilot testing of a panel of samples, including stool and sewage water samples. The outcome of this study will provide critical feedback to support developments in GPLN laboratories across the network.

*Containment of poliovirus and poliovirus laboratories*

The introduction of GAPIII for the containment of poliovirus in 2015 required the implementation and update of existing biorisk management standards in poliovirus laboratories in line with the eradication of wild poliovirus type 2 in September 2015. This revised biorisk management (BRM) system was required to update laboratory standards with new developments in poliovirus containment as the
programme moves ever closer to its eradication goal. During 2015 and 2016, 144 technical staff from all six WHO regions were trained in GAPIII implementation. WHO is also supporting laboratories to comply with BRM standards by providing a tool for gap assessment and support to correct the identified gaps, although progress has been variable across the regions.

To provide further support, WHO has released three guidance papers. Guidance paper 1 describes changes in the handling and storage of type 2 viruses within the GPLN. By 31 July 2016 all polioviruses type 2 (PV2) and biological materials potentially infectious for PV2 should have been destroyed or contained (onsite or after transfer to designated/certified poliovirus-essential facilities). Guidance paper 2 updates polio laboratories on ITD molecular assays and testing algorithms, describing changes in ITD real time RT-PCR assays to inform the GPLN on current and new ITD molecular assays and developments (ITD 4.1, ITD 5.0) and the selection of an additional real-time RT-PCR kit for the ITD assay. Guidance paper 3 covers poliovirus antibody testing for GPLN personnel using dried blood spot, describing the procedure for collecting, packaging, storing and shipping samples collected as dried blood spots for poliovirus antibody testing of personnel in GPLN facilities. This document applies to personnel of GPLN facilities (and other institutional personnel considered to have significant risk of exposure to poliovirus materials) who cannot be tested locally in a certified polio-essential facility, or whose serum samples cannot be shipped to a polio-essential facility of their choice. The determination of poliovirus antibody titres should be done annually.

The global specialized laboratory at NIBSC in the UK shared their experience of introducing and managing GAPIII standards. All poliovirus type 2 work at NIBSC is carried out in a biosafety level (BSL)-3 facility and poliovirus nucleic acid materials are handled at a BSL-2 level
laboratory. NIBSC is planning to certify its poliovirus laboratory as a poliovirus-essential facility to continue working with research and vaccine development projects, followed by the revision of national polio work guidelines. All work-related technical standard operating procedures are in the process of revision to integrate the biosafety and biorisk management components outlined in GAPIII. There are significant challenges to revising existing protocols for the storage and shipping of all polioviruses from the three serotypes, and a lack of guidelines for the management of poliovirus excreters, storage within containment facilities and the use of PV2 for serology assays for seroprevalence, IgG assays, IPV potency testing and outbreak response with mOPV2.

3. Points of action

Coordination between polio laboratories and related public health programmes

- Polio laboratories should prepare detailed inventories of their capacities and assets to share with national health authorities in order to identify common processes and interests to help strengthen cooperation with other public health programmes, including surveillance for vaccine-preventable diseases, such as measles and rotavirus, and other communicable diseases to consolidate transition planning.

Contingency planning for laboratories systems

- Polio laboratories in the Region should develop and establish contingency plans, in coordination with the regional coordinator and Expanded Programme on Immunization/Ministry of Health, to respond to unforeseen increases in workload due to the expansion of activities, a polio outbreak, equipment failure, personal issues and/or non-polio emergencies. These plans should
include the integration of activities undertaken for polio eradication into the overall structures of the institution and to transition staff, as needed. It is recommended to ensure that backup staff are trained on each of the laboratory’s tasks as an integral part of the contingency plan.

**Simulation exercise for laboratory planning and preparedness**

- Polio laboratories should develop and implement periodic simulation exercises to evaluate the level of readiness in emergency situations. Discussions on laboratory methods, results interpretation and reporting for the timely management of resources should be part of these exercises.

**Support for established laboratory methods**

- Laboratories with low workloads should devise a plan for maintaining their ability to perform WHO-accredited laboratory assays. Plans should include the following:
  - laboratories should process and test sufficient samples for poliovirus isolation per year as stated in the WHO accreditation checklist;
  - laboratories should regularly perform complete rRT-PCR ITD/sequencing tests and analysis: at least two samples every two months;
  - laboratories should regularly analyse result files from rRT-PCR ITD/sequencing assays: at least once every month.

- CDC, NIBSC and the Pakistan National Institute of Health should prepare a bank of rRT-PCR ITD/sequencing files for access by laboratories (through the regional/global coordinator) by end
December 2017. The results of these activities should be reviewed during accreditation visits.

- Laboratories in Egypt, Islamic Republic of Iran and Oman should complete implementation of WHO sequencing methods, sharing the results in real-time for timely assessment by CDC experts.
- Polio laboratories should continue to contribute to the development of new methods for use by the GPLN in coordination with the regional coordinator, such as providing potential samples for NGS and pilot testing of new algorithms, when required.

**Quality assurance**

- Laboratories should continue to participate in proficiency panel testing and carefully follow instructions to:
  - adapt standard operating procedures and documents to comply with the changes in WHO-accredited methods such as rRT-PCR ITD assay;
  - implement new recommended methods such as those required for environmental surveillance;
  - review and update the list of standard operating procedures to include those on emergency/contingency plans and spill management. VACSERA may support the regional laboratories by sharing the revised list of standard operating procedures by end of November 2017.

**Laboratory contamination**

- Laboratories should develop a comprehensive and systematic work plan to investigate the root cause of contamination and devise and adopt preventive and corrective measures.
- Appropriate and timely supervision and communication between staff are essential to prevent further incidents.
Data management

- The development of a revised Laboratory Information for Action (LABIFA) data system should be expedited for implementation by the first quarter of 2018.
- The LABIFA system should be updated in real-time to adapt to the changes in poliovirus testing and reporting algorithms.

PID surveillance

- Countries should continue and strengthen PID surveillance through effective coordination and integration with the AFP surveillance network to enhance sensitivity for immunodeficiency-related vaccine-derived poliovirus (iVDPV) detection.
- Any iVDPV isolation should be reported and followed up monthly until two consecutive samples provide negative results.
- Further follow-up samples should be collected later from negative cases and tested at least twice a year since re-infection with iVDPV is frequent.
- Any AFP case with poliovirus isolation having borderline mutations should be followed up and immunological investigations may be requested.
- AFP surveillance staff may be requested to collect contact samples from siblings and close contacts.

Environmental surveillance laboratory workload management

- While establishing or expanding environmental surveillance, careful consideration should be given to the expected increase in laboratory workload and logistical arrangements.
• Laboratory should be part of environmental surveillance planning to prevent any compromise affecting quality of results, especially that of AFP.

• Environmental surveillance data should be utilized to monitor environmental surveillance site selection and performance. Environmental surveillance laboratories should continue to provide their opinion about environmental surveillance site performance based on laboratory findings (rate of non-polio enterovirus and negative samples) to guide the programme on site performance.

• Environmental surveillance laboratories should practice the accreditation checklist when distributed by the regional coordinator before on-site laboratory review.

Establishment of environmental surveillance in countries

• Following provision of WHO support, Iraq and Sudan, being at-risk countries for emergence of VDPV cases, should urgently expedite the establishment of environmental surveillance laboratories to supplement surveillance activities.

Immunization of polio laboratory staff

• All polio laboratories should ensure that staff working with polioviruses are fully immunized with evidence of protective titres, and those who are re-immunized should have their sera tested for antibody titer.

• A single shot of IPV should be given to all staff and sera should be tested 4–6 weeks post-immunization to assess seroconversion results.

• Newly recruited staff should be immunized with one shot of IPV before starting work in the poliovirus laboratory.
Poliovirus containment in the Region

- Poliovirus laboratories, being an essential part of polio containment, should support and continue containment activities to complete Phase 1 (1a and 1b) of poliovirus containment as defined in GAPIII.
- Laboratories should follow WHO guidelines (*Guidance for non-poliovirus facilities to minimize risk of sample collections potentially infectious for polioviruses*) for the containment of potential infectious materials.
- Laboratories should support the national authority for containment by providing technical support for the certification of polio-essential facilities.