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

Muscat, Oman
23–25 May 2016
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1. Introduction

The eighteenth intercountry meeting of directors of national and regional polio reference laboratories in the Eastern Mediterranean Region was held in Oman, Muscat on 23–25 May 2016. The meeting was attended by directors of poliovirus laboratories in Egypt, Islamic Republic of Iran, Jordan, Kuwait, Morocco, Oman, Pakistan, Saudi Arabia, Sudan, and Tunisia. Participants also included scientists from the Centers for Disease Control and Prevention (CDC), United States of America; the National Institute for Biological Standards and Control (NIBSC), United Kingdom; the National Institute of Public Health and the Environment (RIVM), Netherlands; the National Institute for Health and Welfare, Finland; and Kenya Medical Research Institute (KEMRI), along with WHO staff from headquarters and the Regional Office for the Eastern Mediterranean.

Dr Humayun Asghar, Regional Adviser and Regional Polio Laboratory Network Coordinator welcomed the participants and delivered a message on behalf of Dr Ala Alwan, WHO Regional Director for the Eastern Mediterranean. In his message, Dr Ala Alwan commended the excellent work performed by the regional polio laboratory network. He expressed appreciation for the achievements of the polio network laboratories in implementing new methods, techniques and development in poliovirus diagnosis. Following Dr Ala Alwan’s message, H.E. Dr Sultan Al Busaidy, Adviser Health Affairs, Ministry of Health, Oman, welcomed the participants and commended their commitment to polio eradication efforts.

During the meeting the WHO global and regional polio laboratory coordinators presented the current state of the global polio eradication initiative, giving an overview of the performance, activities and challenges faced by WHO network laboratories and describing
planned endgame strategies and current priorities for the programme. The performance of the regional poliovirus laboratory network during the period January 2015 to December 2015 was reviewed. Transmission links between wild polioviruses isolated during that period from both AFP surveillance and supplementary surveillance activities were discussed. Important issues related to the network’s laboratory quality assurance programme were also discussed with the main focus on biosafety aspects related to the work with poliovirus. Performance in the Region continues to be high, with laboratories providing high quality information on poliovirus isolation and characterization in a timely manner which is essential for guiding global polio eradication initiative activities. Laboratories in the Region are also actively engaged in supporting endgame activities such as pilot testing of improved laboratory methods, seroprevalence studies. The laboratory containment activities as per WHO guidelines were also discussed. Recommendations were made to sustain and improve the performance in regional laboratories.

2. Summary of discussions

Status of polio eradication

Encouraged by the 1980 success of global smallpox eradication, the World Health Assembly in 1988 passed a resolution launching the global polio eradication initiative with its goal for the year 2000. At that time, it is estimated that there were >350 000 paralytic polio cases annually in 125 countries. Although the target of eradication by 2000 has long since passed, it great progress has been achieved despite enormous challenges thanks to the efforts of countless polio workers. By 2015 only 74 polio cases were identified in the entire world, 54 in Pakistan and 20 in Afghanistan, all due to wild type 1 polioviruses. Type 2 virus has not been identified since 1999, and type 3 not since
November 2012. The genetic heterogeneity among isolates from Pakistan appears to have decreased between 2014 and 2015, as the number of different genetic clusters in which AFP polio isolates are classified dropped from 7 in 2014 to 5 in 2015 and 1 in 2016 (up to April), which suggests reduced circulation of WPV. But foci of endemic circulation in both Pakistan and Afghanistan continue to delay global polio eradication and to impose the risk of international spread, particularly for countries at risk due to conflict and expanding areas of inaccessibility. The completion of poliovirus eradication in these two endemic countries continues to be jeopardized by lack of safe access to children in conflict-affected areas and inconsistent improvement in the quality and coverage of supplementary immunization activities in key areas. The polio eradication initiative adopted innovative approaches to control poliovirus transmission in endemic areas which include the use of monovalent oral poliovirus vaccine type 1 and monovalent type 3, additionally introduction of short-interval dose campaigns and intense targeted environmental surveillance, which have collectively contribute to a significant decrease in cases.

During 2015, polio cases due to type 2 circulating vaccine-derived polioviruses (cVDPV2s) have been reported from Egypt (n= 1), Islamic Republic of Iran (n=1), Iraq (n=2), and Pakistan (n=4). Though numbers are reduced, the problem of type 2 vaccine-derived polioviruses is persistent. The problem is being addressed by the plan to switch from trivalent oral poliovirus vaccine (OPV) to bivalent (types 1 and 3) in April 2016 and the introduction of a single injection of trivalent inactivated poliovirus vaccine into all national schedules.
Performance of the regional polio laboratory network remains high despite the numerous difficulties and challenges. All regional polio network laboratories are fully accredited and maintain certification-standard performance indicators and efficiently supporting global polio eradication activities. WPVs and vaccine-derived polioviruses (VDPVs) continue to be detected with speed and accuracy despite the rise in workload due to improved AFP surveillance and increased sampling from contacts of AFP cases in infected districts. Also, some laboratories, such as those in Iraq and the Syrian Arab Republic, are performing under critical security situations because of conflict.

The need to devise contingency plans to respond to unforeseen increases in workloads due to a polio outbreak, equipment failure and/or to non-polio emergencies requiring the use of resources from the polio laboratory was discussed. Examples from the WHO regional reference laboratories in Kenya and Egypt were presented. In 2013 the laboratory in Kenya experienced a substantial increase in the workload in a very short period of time due to a WPV1 outbreak in Kenya, Somalia and Ethiopia. The laboratory infrastructure was not able to cope with the testing demand. As a consequence, a backlog of samples for testing accumulated and the laboratory quality indicators dropped, particularly those related to timelines for poliovirus isolation and characterization. Similarly in 2015, samples from Yemen were diverted to KEMRI which resulted in batching and backlog of Yemen samples, ultimately affecting the timeline for poliovirus isolation and characterization. Despite the challenges, the laboratory has maintained good performance over the years to support the programme.

Pakistan and Afghanistan are the only endemic countries in the Region. In Afghanistan, circulation is localized in the southern part of
the country and remaining areas are without any established circulation. The majority of the polio cases in Pakistan are from Khyber Pakhtunkhwa/Federally Administered Tribal Areas (KP/FATA) and Sindh, where campaign quality is compromised due to security and management issues. Environmental surveillance continues in Pakistan and Afghanistan.

The laboratory in Egypt has been an excellent performer for a number of years and has shown a dramatic increase in workload in recent years, as apart from acting as a national polio laboratory for Egypt. VACSERA laboratory is a reference laboratory providing virus isolation and intratypic differentiation (ITD) facility to Iraq, Lebanon, Sudan, Syrian Arab Republic and Yemen. Environmental surveillance was introduced in 2001 and is well established with 42 sample collection sites covered all of the 27 governorates. Additionally, the Regional Commission for Certification of Poliomyelitis Eradication (RCC) in its 27th meeting in April 2014 recommended upgrading VACSERA for genomic sequencing of wild poliovirus. Training was conducted with the support of the Regional Office, USAID, CDC and NIH Pakistan for establishment of third nucleotide sequencing laboratory in the Region after Pakistan and Tunisia. Furthermore, the national polio laboratory of Saudi Arabia was upgraded for ITD/VDPV rRT-PCR assay after conducting the training with support of NIH Pakistan.

*Virus surveillance*

The WHO regional reference laboratory in Pakistan continued to maintain a high level performance during 2015. Laboratory quality indicators such as reporting of cell culture results within 14 days and ITD results within 7 days were well above the minimum requirements from both Pakistan and Afghanistan. Stool samples from 5648 AFP
cases and 1875 contacts were received from Pakistan during 2015. A total of 54 WPV1 and two cVDPV2 and two aVDPV2 cases were identified. A total of 20 WPV1 cases were confirmed from stools of 2704 AFP cases and 1230 contacts from Afghanistan. The non-polio enterovirus (NPEV) isolation rate was 23% and 22% for Pakistan and Afghanistan, respectively. 438 environment samples from 40 different sites in Pakistan were also analysed. Out of these, 83 samples were shown to contain WPV1 strains and 13 were positive for cVDPV2 isolates. Environment surveillance in Afghanistan was also initiated at the end of 2013 from three different collection sites, which were then increased to 14 sites in 2015. 148 environmental sewage samples from Afghanistan were received from these sites during 2015. Out of these, 19 samples were positive for WPV1 and no VDPV have been isolated.

Molecular epidemiology

All WPV1 isolates from Pakistan and Afghanistan belong to the SOAS genotype. All cases have been detected from the Quetta, Karachi, northern Sindh and Khyber Pakhtunkhwa blocks showing persistent circulation of WPV. The significant decrease in cases is attributable to the use of monovalent oral poliovirus vaccine type 1 and monovalent type 3, the introduction of short-interval dose campaigns and intense and targeted environmental surveillance. The genetic heterogeneity among isolates from Pakistan and Afghanistan appears to have decreased slightly in 2015 as viruses from 6 genetic clusters were detected in Pakistan, compared to 7 genetic clusters in 2014. Similarly, in Afghanistan, a significant reduction in genetic diversity occurred during 2015 and the number of genetic clusters decreased to 2, compared to 6 distinct genetic clusters in 2014. Population migration is still regarded as the major source for the transmission of WPVs between different areas in both countries. The core reservoirs remained active including southern Afghanistan.
(Helmand) and eastern Afghanistan (Nangarhar) with persistent WPV circulation that needs urgent attention.

In Pakistan, core reservoirs including Khyber-Peshawar, Quetta Block, Karachi and northern Sindh were active and transmission within these reservoirs remains intense, as shown by sustained local circulation and continuous reseeding of non-reservoir areas. The most intense is Khyber-Peshawar-Nangarhar circulation because of frequent movement of people across the border. Quetta Block has been re-infected from two-way importations (Khi-Kandahar). Northern Sindh with sustained WPV1 transmission of 3 different clusters is of great concern, showing low-level transmission as viruses have been detected in environmental sewage samples. Ongoing low-level circulation in Karachi also remains a concern, but an equal concern is the continuous risk of widespread dissemination.

**Vaccine-derived polioviruses**

The first emergence of a cVDPV outbreak in Afghanistan and Pakistan was recorded in 2009. Up until the end of 2015, two type cVDPV and two type aVDPV have been identified in Pakistan (Khyber, Hangu and Karachi Gulberg).

The national polio laboratory of the Islamic Republic of Iran detected 15 iVDPVs in AFP cases (1 iVDPV1, 11 iVDPV2, 1 iVDPV3 and 2 iVDPV1+iVDPV2 cases) since 1995. Further investigation showed that all these cases had primary humoral immunodeficiency (PID). Five of the patients are still alive and have stopped poliovirus shedding. The last case was detected in 2015 in a 6 month-old female with severe combined immune deficiency (SCID). The laboratory received 4 specimens from this case, all positive for iVDPV2. The patient died due to immunodeficiency complications. All four contact
specimens of this case were found negative. Additionally, VACSERA laboratory Egypt reported two type 2 VDPV cases in 2015.

Environmental surveillance

Environmental surveillance is used to supplement AFP surveillance, sampling populations rather than individuals. It represents a powerful tool to detect wild or VDPV circulation and to guide further surveillance activities and immunization campaigns. The current sewage concentration method recommended by the WHO global poliovirus laboratory network is the two-phase separation. There are other methods described in the literature, and laboratories of the global poliovirus laboratory network are actively testing different techniques with a view to complete WHO guidelines that will include detailed standard methods and accreditation procedures to ensure quality assurance.

Afghanistan, Egypt and Pakistan have implemented environmental surveillance to detect polioviruses in sewage water in strategic locations. The regional reference laboratory in Egypt was a pioneer in implementing this technique. It started with 2 provinces, 5 sites and 5 samples per month and now in 2015, environmental surveillance for poliovirus is expanded to all provinces of Egypt. The the monthly number of sewage specimens is 42–44 samples from 42 sites in 27 governorates.

Since 2009, environmental surveillance is being conducted in strategic locations in Pakistan to detect polioviruses in sewage water samples. Currently, 40 active environmental surveillance sites are operating in 16 districts/towns across 4 provinces.
A total of 438 samples were tested during 2015, 56% of them were shown to contain Sabin viruses, 19% WPV isolates, 22% NPEV and 3% VDPVs. Environmental surveillance was initiated in Afghanistan in 2013 in two cities of Kandahar (Khandak and Rarobot). Currently, there are 14 active sites in 6 districts/towns. A total of 148 samples were tested during 2015; 56% of them were shown to contain Sabin viruses, 13% WPV isolates and 30% NPEV.

Laboratory quality assurance

Annual proficiency testing and assessment of laboratories continue to be critical for quality assurance of the performance in polio laboratories. Four different proficiency testing panels are in use for evaluating: a) accuracy of virus isolation; b) ITD by real-time PCR (rRT-PCR); c) rRT-PCR for VDPV screening; and d) sequencing poliovirus isolates. The proficiency testing programme is coordinated by WHO in collaboration with the global specialized laboratories in the United States and the Netherlands. A standardized test to measure the sensitivity of the cell lines for poliovirus infection is also required for laboratories to be accredited. At the time of the meeting, all laboratories had passed the most recent proficiency tests for all relevant laboratory techniques while awaiting the results of proficiency panel of sequencing.

Regional laboratories that participated in the virus isolation proficiency panel scored 100%. Three laboratories could not yet be provided with the virus isolation proficiency panel (Iraq, Sudan and Syrian Arab Republic). The biggest issue was in interpretation of rRT-PCR ITD/VDPV data throughout the regional laboratory network. There is need to harmonize the terminology of final results reporting, especially for discordant results in the laboratory network. Regional laboratories are highly proficient in polio PCR assays, turnaround time
is excellent for all laboratories and proficiency test results correlate with routine results.

3. Points for action

Contingency planning for laboratory systems

- Laboratories should finalize contingency plans to respond to unforeseen increases in workloads due to a polio outbreak, equipment failure and/or non-polio emergencies.
- The regional coordinator should assess capacity for virus isolation/ITD/sequencing to identify resources across the Region.
- The regional coordinator should prepare a template to include elements required for contingency planning (current capacity, possible networking between laboratories, chains of communication).

Biorisk management systems

- Heads of laboratories should prepare written plans which should include risk assessment for handling PV2 infectious materials post-OPV switch following WHO guidelines. Plans should cover:
  - Processing of samples: all WHO-accredited network laboratories can process stool and environmental samples and assess for the presence of poliovirus following recommended protocols. However, laboratories not certified as PEF cannot further work with those materials once a PV2 has been identified.
  - Transferring of PV2 infectious materials to PEF laboratory: arrangement should be made to immediately ship PV2-positive samples to PEF for further analysis.
Management of storage and destruction: laboratories should not work with PV2 material once identified and should keep all PV2 materials (stool, supernatant, isolate) in secure place until transferring to PEF followed by destruction of any remaining PV2 materials by recommended procedures and with full documentation of the process in consultation with the WHO regional polio laboratory coordinator.

Laboratories should destroy all historical PV2 materials including PV2 LQC and NIBSC reference standards before June 2016 following recommended procedures and with full documentation of the process.

- WHO should sustain the cohort of IATA-certified Infectious Substance Shipping Training (ISST) shippers in the polio laboratories through web-based refresher courses or onsite training of new staff.
- WHO should map couriers in different countries/regions to facilitate shipping of AFP and environmental surveillance samples.
- Biorisk management systems should be reviewed during accreditation visits.

Laboratory capacity to adapt to new diagnostic/containment requirements

- Current plans for laboratories to acquire new capacities should be implemented.
  - Islamic Republic of Iran: environmental surveillance
  - Saudi Arabia: environmental surveillance
  - Sudan: environmental surveillance + ITD
  - Jordan: environmental surveillance + ITD
- WHO will assist with arranging logistics, training, validation and follow-up.
• Human resources should be provided by national governments.
• WHO to organize an inter-regional workshop for sequencing to be held in Oman in the fourth quarter of 2016. Following this laboratories in the Islamic Republic of Iran, Kuwait, Oman and Saudi Arabia and will be assessed to implement this technology.

Support for established laboratory methods

• Laboratories with low workloads should devise a plan for maintaining the ability to perform WHO-accredited laboratory assays:
  – 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, NIH Pakistan should prepare a bank of rRT-PCR ITD/sequencing files for access by laboratories (through the regional/global coordinator). Results of these activities should be reviewed during accreditation visits.
• RIVM/CDC should prepare full reports of annual proficiency panel testing describing characteristics of samples, interpretation of results, laboratory errors.
• NIBSC/Iranian PL should review and follow up on issues with Pan-PV rRT-PCR ITD reaction using the Rotor-Gene platform.
• WHO should assist laboratories with the purchase of sequencing analysis software licence(s) as required.
Quality assurance

- Laboratories to review cell banks.
  - All laboratories in the Region should prepare a comprehensive review of master, working and distributing cell banks (if applicable) available in the laboratory with details of storage location, origin, passage history, records of preparation, data from any cell sensitivity, cell authenticity, mycoplasma testing, etc.
  - Cell banks should be harmonized.
  - Laboratories should be aware that NPEV can produce CPE in RD cells different to that typically observed for PV. Laboratories should record these observations including photographs if possible and share with the regional coordinator.

- Laboratories to update quality assurance procedures.
  - Laboratories should be aware of the continuous evolution of WHO-accredited laboratory methods such as rRT-PCR ITD and adapt quality assurance procedures (SOPs, forms, training, troubleshooting, biosafety/biosecurity, etc.) accordingly.
  - Laboratories performing environmental surveillance should adopt recommended procedures, comply with requirements in the WHO accreditation checklist and perform proficiency testing following WHO guidelines.

Environmental surveillance in the Region

- The regional coordinator should review the capacity for environmental surveillance in the Region and devise further plans for expansion following WHO guidelines.
- Evaluation of the bag mediated filtration system should be completed and data should be carefully analysed keeping in view the programme priorities and practical issues related to implementation in the national poliovirus laboratory network.

**GAPIII Phase 1 containment of poliovirus**

- The national poliovirus laboratories should support the GAPIII Phase 1 containment activities and work closely with national containment coordinators/task force.

**Data management**

- The revised version of LABIFA should be implemented in all laboratories by the end of 2016.
- Data in .rec files from AFP surveillance and the polio laboratory should be harmonized in order to optimize the use of LABIFA.
- The LABIFA database should be updated in real time to adapt to changes in diagnostic requirements.
- All laboratories should provide regular feedback on their experience with the LABIFA system.