

Summary report on the

Sixteenth intercountry meeting of directors of poliovirus laboratories in the Eastern Mediterranean Region

WHO-EM/POL/407/E

Muscat, Oman
18–21 November 2013



**World Health
Organization**

Regional Office for the Eastern Mediterranean

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1. Introduction

The sixteenth intercountry meeting of directors of national and regional polio reference laboratories in the Eastern Mediterranean Region was held in Muscat, Oman from 18 to 21 November 2013. Directors of poliovirus laboratories in Egypt, Islamic Republic of Iran, Iraq, Jordan, Kuwait, Morocco, Oman, Pakistan, Saudi Arabia, Sudan, Syrian Arab Republic and Tunisia attended the meeting. Participants also included a scientist from the Centers for Disease Control and Prevention (CDC), United States of America, the National Institute for Biological Standards and Control (NIBSC), United Kingdom, National Institute of Public Health and the Environment (RIVM), the Netherlands, and Kenya Medical Research Institute (KEMRI), along with staff of WHO headquarters and the Regional Office for the Eastern Mediterranean.

The meeting reviewed the current status of the global polio eradication initiative giving an overview of the performance, activities and challenges faced by the regional poliovirus laboratory network and describing planned endgame strategies and current priorities for the programme. The Eastern Mediterranean Region presents important challenges as the home of two of the three countries in the world that have never eliminated indigenous wild poliovirus (WPV) transmission. As well, there is a large outbreak in Somalia and new polio cases have been found in the Syrian Arab Republic due to the deterioration of public health services. The performance of the regional poliovirus laboratory network during the period January 2012 to September 2013 was reviewed. Transmission links between wild polioviruses (WPVs) isolated during that period from both acute flaccid paralysis (AFP) surveillance and supplementary surveillance activities were discussed. 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. Important issues related to the regional network's laboratory quality assurance programme were also discussed, with the main focus on biorisk aspects related to the work with poliovirus. An introductory workshop on biorisk management in poliovirus laboratories was conducted for the group. The aim was to qualify trainers who can run the campaign in their respective countries. Some concerns remain in some aspects of laboratory work, particularly when laboratories are required to handle high workloads unexpectedly as a consequence of a polio outbreak or increased surveillance activities. Recommendations were made to sustain and improve the performance in regional laboratories.

2. Summary of discussions

Status of polio eradication

Global: The world is closer than ever to global polio eradication. The number of cases of paralytic poliomyelitis is low; there have been no polio cases due to WPV type 2 or WPV type 3 since October 1999 and November 2012, respectively; and India, where large epidemics were frequent until recently, has not seen a polio case since February 2011. However, several concerns and challenges remain. Three countries, Nigeria, Afghanistan and Pakistan, have still not been able to interrupt circulation of indigenous WPV type 1 (WPV1). There is also a large outbreak due to WPV1 in Kenya, Ethiopia and Somalia and small outbreaks due to type 2 circulating vaccine-derived polioviruses (cVDPV2) continue to occur in parts of Central Africa and the Middle East. Furthermore, WPV1 strains related to viruses circulating in Pakistan were found in environmental samples in Israel from February to December 2013 indicating widespread virus circulation in a

population with >95% vaccine coverage with inactivated polio vaccine (IPV) and the absence of known paralytic cases. WPV1 isolates also related to the same genetic cluster from Pakistan have been detected in the Syrian Arab Republic in late 2013 causing at least 13 cases of paralytic disease. The main reason for this appears to be the fact that there are insufficient surveillance activities in some of those countries and in some cases, the existence of deficient public health services that can result in interruption of immunization activities and poor public health assistance in general.

Regional: As stated above, the Region presents important challenges as the home of two (Pakistan and Afghanistan) of the three countries in the world that have never eliminated indigenous WPV transmission. There is also a large polio outbreak due to WPV1 ongoing in Somalia as immunization activities are seriously compromised due to continuing civil unrest and inability to access certain areas, although the epidemic appears to be declining. The Syrian Arab Republic, with serious deterioration of public health services due to the long civil war, has also recently reported a number of confirmed polio cases due to WPV1 strains genetically linked to viruses found in sewage samples from Egypt, Israel and Pakistan. As a consequence, the number of polio cases in the Region has multiplied by 3 in 2013 and cases in the Region now account for 75% of global polio cases.

The genetic heterogeneity among isolates from Pakistan appears to have decreased between 2010 and 2013, as the number of different genetic clusters in which AFP polio isolates are classified dropped from 11 in 2010 to 5 in 2012 and 3 in 2013, which suggests reduced circulation of WPV. However, more than 500 000 children were still missed in oral polio vaccine (OPV) campaigns in 2013 in Federally Administered Tribal Area (FATA) and Khyber Pakhtunkhwa (KP),

where 86% of polio cases occurred. Local bans on immunization, military operations and intimidation of and attacks on polio volunteers continue to be major challenges in these areas.

Polio laboratory network performance

Performance of the regional polio laboratory network remains high despite the numerous difficulties and challenges. All laboratories in the Region 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 Syria, are performing under dangerous security situations. During 2012, 94% of isolations of polioviruses and 99% of intratypic differentiation (ITD) results in the regional laboratories were obtained within the required timelines. A total of 25 603 specimens from all surveillance activities were processed. Of those, 20 998 were faecal specimens from cases of acute flaccid paralysis. All laboratories using rRT-PCR for ITD have now revised the technique and adopted the dual-stage protocol. Plans of action for the implementation of the rRT-PCR ITD method are in place in Saudi Arabia and the Syrian Arab Republic. Environmental surveillance continues in Egypt and was expanded in Pakistan and initiated in Afghanistan in 2013. The Region continues to recognize the importance of VDPV isolation and network laboratories in Egypt, Islamic Republic of Iran and Tunisia are participating in projects to assess poliovirus excretion by immunodeficient patients.

Virus surveillance

Pakistan and Afghanistan: Laboratory quality indicators in 2012 and 2013 such as cell culture results reported within 14 days or ITD results reported within 7 days were well above the minimum requirements from both Pakistan and Afghanistan. In 2012, a total of 58 WPV cases (55 WPV1, two WPV3, and one WPV1/WPV3 mixed infection) were reported in Pakistan compared with 198 cases (196 WPV1 and two WPV3) in 2011, a 71% decrease. Fifty-two WPV cases were reported during January–September 2013, compared with 54 cases during the same period in 2012. From August 2012–September 2013, 52 cVDPV2 cases were detected, including 30 cases (58%) identified in FATA during January–September 2013. Environmental surveillance for polioviruses is routinely conducted in 23 sites in 11 cities in all major provinces of Pakistan. In 2012–2013, WPV1 were isolated in nearly all samples in Peshawar (KP) and Hyderabad (Sindh), but the frequency of isolation declined in other sites. In 2013, acute flaccid paralysis (AFP) and environmental surveillance suggest that WPV1 transmission has been restricted to high-risk areas of FATA and KP and WPV1 circulation has apparently been interrupted in the Quetta block, Baluchistan, one of the historic reservoir areas. WPV3 has not been detected in any stool or sewage sample in Pakistan for more than one year.

During 2012, 37 WPV1 cases were confirmed in Afghanistan, compared with 80 cases in 2011; nine WPV1 cases were confirmed during January–September, 2013, compared with 26 WPV1 cases during the same period in 2012. Since November 2012, no WPV1 cases have been reported from the southern region, previously the main WPV reservoir in Afghanistan. All nine polio cases in 2013 were in the eastern region. From October 2012 to March 2013, 14 polio cases caused by cVDPV2s were detected in the southern region.

Outbreak in Syria: One of the major concerns in the Region is the recent detection of WPV1 circulation in Deir Al Zour. A sudden surge of AFP cases was detected in Deir Al Zour in September–October 2013. The number of confirmed polio cases to date is 13, all associated with WPV1 isolates related to WPV1 genetic cluster R3 originally found in Pakistan. All major AFP surveillance indicators such as the number of AFP cases detected and the percentage of adequate stool samples collected from AFP cases have declined significantly in the past few years, from 194 in 2010 to 109 in 2012 and from 84.4% in 2012 to 72.7% in 2013, respectively. The last case due to indigenous WPV1 was detected in the Syrian Arab Republic in 1995.

Somalia and South Sudan: A massive outbreak due to WPV1, which is still ongoing, was detected in Kenya, Somalia and Ethiopia in 2013. CDC confirmed the isolates as WPV1 genetically related to viruses first seen in Nigeria. The outbreak subsequently spread to other areas such as eastern Kenya, elsewhere in Somalia and the Somali region of Ethiopia. This has caused major impact in the laboratory in KEMRI, which handles all AFP samples from the three countries as well as those from South Sudan, as there has been a substantial increase in the workload in a very short period of time. 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. This difficult situation was likely one of the main reasons that led to the cross-contamination of stool samples from South Sudan which were initially identified as positive for WPV1 genetically linked to outbreak strains. This incited major concern as it was thought that the WPV1 outbreak had expanded to a much wider area. However, a thorough investigation

including nucleotide sequence comparison between virus isolates obtained in the same laboratory and scrutiny of laboratory records clearly identified the virus isolates from South Sudan as cross-contamination from samples from Somalia that had been handled in the same laboratory area at the same time.

Molecular epidemiology

WPV: All WPV1 isolates from Pakistan and Afghanistan belong to the SOAS genotype. Six genetic clusters were represented in AFP cases and environmental specimens in 2012, R2B, R3A, R3B, R4A, R4B and R5. In Pakistan, local virus circulation occurred in all areas where WPV was found. Cross-border transmission to Afghanistan occurred in the south (from Quetta block) and in the north (from FATA). The majority of WPV1 isolates in Pakistan were found in FATA. In Sindh, one virus from cluster R2B was found and one virus from cluster R4B was isolated in the provincial border with Baluchistan. In KP, many R4B cluster viruses were found. Two R4B cluster viruses were isolated in Northern Punjab. Viruses from all 3 clusters found in AFP cases plus strains from cluster R3A in Sindh and Punjab were found in environmental samples. Most environmental isolations correlated with AFP case distribution. Viruses from cluster R4A were isolated from environmental samples in Baluchistan near the border with Afghanistan. All WPV1 isolates from 2013 in Somalia were from WEAF-B1 cluster N5A originally found in Nigeria.

Vaccine-derived polioviruses: Two related circulating VDPV2 (cVDPV2) outbreaks occurred in Pakistan and Afghanistan between 2009 and 2013 with emergence in Nade Ali Helmand and circulation between 2009 and 2013 and a second emergence in Killa Abdullah and circulation between 2012 and 2013. The last case of cVDPV reported in Afghanistan was in March 2013. All cases in Afghanistan

were reported from two south provinces Helmand (9 cases) and Kandahar (5 cases). In Pakistan, there is a large cVDPV2 outbreak in North Waziristan with emergence of a new genetic lineage. Immunodeficient VDPV (iVDPV) isolates from AFP samples of 12 immunodeficient patients were identified in the Islamic Republic of Iran between 1995 and 2013. Samples corresponded with 10 males and 2 females, 5 cases with Severe Combined Immunodeficiency (SCID), 3 with X-Linked Agammaglobulinemia (XLA) and 2 others with unknown disease. Two isolates from two immunodeficient patients in Iraq were confirmed as VDPV2 by CDC. No VDPVs were detected in 2012–2013 from AFP surveillance. In 2012, 8 ambiguous VDPV (aVDPV) isolates were found in environmental samples, 7 aVDPV2s and 1 aVDPV1, from 5 sites in Giza, Ismailia, Alexandria, Cairo and Beni Suef. Three aVDPV2s were found in 2013 from sites in Cairo, Minya and Fayoum. Two patients were found to be excreting iVDPVs in 2011, one iVDPV1 from Behiera and one iVDPV2 from Cairo through an iVDPV surveillance project in Egypt.

Environmental surveillance

Environmental surveillance has been 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. Plans are in place to add at least 15–20 additional sampling cities/sites in potential first line importation countries by end 2015. Afghanistan, Egypt and Pakistan have implemented environmental surveillance to detect polioviruses in sewage water in strategic locations. In Pakistan, currently, 23 environmental surveillance sites have been designated in 11 districts/towns that cover all the provincial capitals and regional hubs. During 2012–2013, WPV1 was isolated in nearly all samples in Peshawar (KP) and Hyderabad (Sindh) but the frequency of isolation

declined in other sites. The latest WPV3 isolated from a sewage sample was collected in Karachi in October 2010. In Egypt, environmental surveillance has played an important role in the eradication of WPV. In December 2012, WPV1 was isolated from two sites in Cairo (Alsalam and Alhaggana). The virus was genetically linked to WPV circulating in Sindh, Pakistan, during 2012.

Laboratory quality assurance

The annual proficiency testing and assessment of laboratories continue to be critical for the quality assurance of the performance in polio laboratories. Six different proficiency testing panels are in use for evaluating: a) accuracy of virus isolation; ITD by b) ELISA, c) probe hybridization, d) traditional PCR, e) real-time PCR (rRT-PCR), and f) rRT-PCR for VDPV screening, g) 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. In 2013, all laboratories passed proficiency tests. Changes in accreditation checklists are planned to streamline the accreditation process. This is because there are some chapters of the accreditation checklists that are no longer applicable and there is confusion in the calculation of some scores. The new changes will hopefully reduce the subjective interpretation/scoring during assessment of laboratory work practices and solve frequent shared concerns on inaccuracies/inconsistencies reported by on-site reviewers.

Guidelines for troubleshooting rRT-PCR ITD results have been given by CDC to solve problems that the laboratory may encounter during testing of poliovirus isolates. Common issues include obtaining amplification results with anomalous curves and forgetting to deselect the ROX reference dye. The use of the dual-stage rRT-PCR ITD

protocol increases sensitivity more than tenfold and generally improves interpretation due to more robust amplification curves.

Development and evaluation of new diagnostic methods and reagents

Regional reference laboratories and particularly global specialized laboratories continue to contribute to the validation, implementation and improvement of methods used by the global poliovirus laboratory network. This is particularly relevant in the context of planned changes for the post-eradication and post-OPV eras which will include a switch from trivalent OPV to bivalent OPV to monovalent OPV, the introduction of global IPV use and, eventually, the total interruption of OPV immunization. Scientists from CDC are continuously updating protocols for ITD rRT-PCR to help increasing the sensitivity and specificity for detection of WPVs and VDPVs. Future developments in ITD rRT-PCR testing will include a quadruplex assay to detect Sabin 1, 2 and 3 + enteroviruses in the same reaction. Primers and probes are also being developed and tested for the detection of WPVs currently circulating in the African and the Eastern Mediterranean regions.

Biorisk management

An interactive condensed workshop was conducted to introduce the new concept of biorisk management, which combines risk assessment, risk mitigation, and performance systems. The purpose of the workshop was for participants to understand key principles of biorisk management including risk assessment, risk mitigation and performance management. It was targeted at polio laboratory directors and gave participants an opportunity to learn and use a robust methodology to identify and control the biosafety and biosecurity risks of bioscience laboratories. It was conducted as group work, case

studies and scenarios, demonstrations, hands-on activities, question-and-answer sessions, in highly interactive way

3. Recommendations

1. Maintaining high levels of performance by laboratories of the regional polio laboratory network continues to be a priority in a context of the current political situation, competing public health priorities and budget constraints. To achieve this:
 - 1.1 The Regional Office should continue to work closely with governments and donors to ensure adequate logistics, human and financial resources are available for the regional laboratory network.
 - 1.2 Regional and national reference laboratories should maintain frequent communication between themselves and the Regional Laboratory Coordinator to help monitoring performance, facilitate shipment of poliovirus isolates for ITD characterization and ensure correct reporting of results and follow-up actions.
 - 1.3 Laboratories should consider the use of FTA cards for shipment of isolates/RNA when shipment of virus is challenging following a validation process which requires sending both virus isolates and FTA cards in parallel for a set period.
 - 1.4 The Regional Laboratory Coordinator and laboratory directors should work together to identify expertise available in network laboratories that can be used in other global health programmes as part of the global poliovirus laboratory network legacy management system in preparation.

2. Polio network laboratories should continue to provide the global polio eradication initiative with high quality data on poliovirus isolation and characterization from AFP and supplementary surveillance activities in a timely manner. To achieve this:
 - 2.1 Laboratories should continue to use methods that have been standardized and validated by WHO and devise standard operating procedures accordingly.
 - 2.2 The interpretation of molecular epidemiological data generated by laboratories should be coordinated by Regional/Global Laboratory Coordinators and scientists from the global specialized laboratories to ensure that accurate chronological geographical are established between poliovirus isolates from different areas, country or regions as this provides essential information to guide global polio eradication activities.
3. Laboratories are encouraged to participate in iVDPV surveillance projects and should provide relevant data VDPV isolates from all surveillance activities compiling, analysing and sharing this information with the national polio eradication programme.
4. Laboratory directors should prepare, in coordination with the Regional Laboratory Coordinator and national authorities, a contingency plan to respond to unforeseen increases in workloads due to a polio outbreak and/or to an increase in surveillance activities. To achieve this:
 - 4.1 The contingency plan should include all aspects that contribute to successful laboratory performance and might have an impact during emergencies such as human resources,

facility, supplies, testing strategy, data management, communication, coordination, funding and biorisk issues.

- 4.2 Each Laboratory Director should continuously review all processes involved in laboratory performance to be able to identify quickly any weaknesses due to the increased activities which can lead to inadequate performance and misinterpretation of critical results.

5. There is a need to improve standardization procedures and data analysis for environmental surveillance activities conducted in the Region as this method is known to be very sensitive in detecting poliovirus circulation even in the absence of known paralytic cases. To achieve this:
 - 5.1 The Regional Office should provide laboratories with revised guidelines for environmental surveillance defined by WHO.
 - 5.2 Laboratories should replace NAPH with the rRT-PCR ITD method for the characterization of poliovirus isolates from environmental samples.
 - 5.3 The Regional Office and regional polio laboratories should work together to increase the quality of data from supplementary surveillance activities making full use of available reporting systems for this purpose.
 - 5.4 Laboratories should define and arrange requirements for testing environmental samples when the implementation/expansion of this technique is required by the country without compromising the timely testing of AFP samples.

6. Maintaining high levels of laboratory quality assurance according to the WHO accreditation process is essential. To achieve this:

- 6.1 Laboratories should review the new proposed checklists for accreditation which are procedure-specific as each laboratory process requires different evaluation criteria and provide feedback to the Regional Laboratory Coordinator.
 - 6.2 Laboratories should maintain hardcopies of training records/CVs for all staff members. Records should include demonstration of competence in laboratory techniques, academic records, training in biosafety, etc.
 - 6.3 All queries/troubleshooting related to specific laboratory methods should be addressed to scientists in global specialized laboratories as instructed by the Regional Laboratory Coordinator.
 - 6.4 Laboratories wishing to implement the rRT-PCR ITD and/or sequencing WHO methods should follow an appropriate implementation/validation process as instructed by the Regional Coordinator.
 - 6.5 Laboratories with low workloads should ensure that a minimal number of samples are tested regularly with all methods for which they are accredited.
7. Improving the quality of laboratory work with cell cultures should be an immediate priority. To achieve this:
 - 7.1 Laboratories should review their training procedures and standard operating procedures to ensure that they are fit for purpose and follow WHO recommendations.
 - 7.2 Laboratories should perform cell sensitivity testing regularly according to WHO recommendations and standard operating procedures. Laboratory directors, with support from associated regional reference laboratories, should ensure that cell sensitivity results are properly evaluated and that any necessary corrective actions are promptly taken.

- 7.3 Results from cell sensitivity assays should be critically evaluated during the accreditation process with clear conclusions and recommendations if needed.
8. Training for biorisk management and promoting biosafety practices in all laboratories should be a high priority for the Region. To achieve this:
- 8.1 Laboratory directors should consider implementation of a biorisk management system in their laboratories, in coordination with local and national authorities and in collaboration with WHO.
 - 8.2 The biorisk management system should include a clear definition of roles and responsibilities related to biorisk and biosafety practices.
 - 8.3 Laboratory containment activities should continue as required by WHO guidelines and priorities established by the global polio eradication endgame strategies.



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