World Health Organization
REGIONAL OFFICE FOR Europe

WHO EUROPEAN REGIONAL POLIO LABORATORY NETWORK

Meeting report

29–31 August 2017
Copenhagen, Denmark

ABSTRACT

Representatives from 46 laboratories in 36 countries in the WHO European Regional Polio Laboratory Network met together with representatives from WHO Geneva and international partner agencies in Copenhagen, Denmark from 29 to 31 August 2017. The main objectives of the meeting were to review and discuss the current status of the Global Polio Eradication Initiative (GPEI), activities and status of the Global and Regional Polio Laboratory Networks, implementation of recently introduced testing algorithms, and new laboratory tools and approaches in development. A particular objective was to review requirements and implementation of laboratory containment requirements following the successful withdrawal of trivalent oral polio vaccine (tOPV) in April 2016 and the subsequent requirement for laboratory containment of Sabin-related type 2 polioviruses.

KEYWORDS

POLIOMYELITIS – diagnosis, epidemiology, prevention and control
POLIOVIRUS VACCINE – administration and dosage
POLIOVIRUS CONTAINMENT
LABORATORIES
EPIDEMIOLOGICAL MONITORING

Address requests about publications of the WHO Regional Office for Europe to:

Publications
WHO Regional Office for Europe
Marmorvej 51
DK - 2100 Copenhagen Ø, Denmark

Alternatively, complete an online request form for documentation, health information, or for permission to quote or translate, on the Regional Office website (http://www.euro.who.int/pubrequest).

Contents

Abbreviations 1

Summary 3

Introduction 3

Day 1. Laboratory Network 3

Session 1. WHO polio laboratory network - global and regional updates 3

Session 2. External quality assurance - proficiency testing (PT) 8

Session 3. Developing strategies for the future 10

Session 4. Investigating samples of programmatic importance 12

Day 2. PV. Challenges of containment breach and risk assessment 12

Session 1. Global and Regional updates on PV containment progress 12

Session 2. Sharing experience and progress on PV containment implementation 15

Session 3. Containment breach and response procedures 18

Day 3. New tools to address laboratory and containment issues 20

Session 1. New containment inventory and LDMS 20

Meeting summary 20

Recommendations 23

Annex 1. List of participants 25

Abbreviations

ABI	Applied Biosystems Inc
AFP	acute flaccid paralysis
bOPV	bivalent oral polio vaccine
BRM	biorisk management
BSL-3	Biological Safety Level 3
CAG	Containment Advisory Group
CCS	Containment Certification Scheme
CDC	Centers for Disease Control and Prevention, US
CMG	WHO Containment Management Group
CP	Certificate of Participation
cVDPV2	circulating vaccine-derived poliovirus type 2
DTT	dithiothreitol
FTA card	Flinders Technology Associates filter paper
GAPIII	The WHO Global Action Plan to minimize poliovirus facility-associated risk after type-specific eradication of wild polioviruses and sequential cessation of oral polio vaccine use
GCC	Global Certification Commission
GCC-CWG	Global Certification Commission Containment Working Group
GPEI	Global Polio Eradication Initiative
GPLN	Global Polio Laboratory Network
IPV	inactivated polio vaccine
ITD	intratypic differentiation
LDMS	Laboratory Data Management System
LED	Light Emitting Diode
mOPV2	monovalent polio vaccine type 2
NAC	National Authority for Containment
NCC	national certification committee
NIBSC	National Institute for Biological Standards and Controls
NPCC	National Poliovirus Containment Coordinator
NPEV	non-polio enteroviruses
PCR	polymerase chain reaction
PEF	poliovirus-essential facility
PHAS	Public Health Agency of Sweden
PHR	Public Health Response
PID	primary immunodeficiency
PIM	potentially infectious materials
POSE	polio outbreak simulation exercise
PT	proficiency testing
PV	poliovirus
PV2	poliovirus type 2
RCC	Regional Certification Commission for Poliomyelitis Eradication
RIVM	National Institute of Public Health and the Environment, the Netherlands
RNA	Ribonucleic acid

rRT	reverse transcription reaction
SIA	supplementary immunization activities
SOP	Standard Operating Procedure
SWOT analysis	strengths, weaknesses, opportunities and threats analysis
tOPV	trivalent oral polio vaccine
UK	United Kingdom
VDPV	vaccine-derived poliovirus
VP1	viral protein 1
WHA	World Health Assembly
WHO	World Health Organization
WHO HQ	World Health Organization headquarters
WPV	wild poliovirus
WPV1	wild poliovirus type 1
WPV2	wild poliovirus type 2
WPV3	wild poliovirus type 3

Summary

Representatives from 46 laboratories in 36 countries in the WHO European Regional Polio Laboratory Network met together with representatives from WHO Geneva and international partner agencies in Copenhagen, Denmark from 29 to 31 August 2017. The main objectives of the meeting were to review and discuss the current status of the Global Polio Eradication Initiative (GPEI), activities and status of the Global and Regional Polio Laboratory Networks, implementation of recently introduced testing algorithms, and new laboratory tools and approaches in development. A particular objective was to review requirements and implementation of laboratory containment requirements following the successful withdrawal of trivalent oral polio vaccine (tOPV) in April 2016 and the subsequent requirement for laboratory containment of Sabin-related type 2 polioviruses. While laboratory containment of these materials is progressing, its implementation is behind the planned timeline and there is an urgent need to accelerate activities. Mechanisms for the certification of poliovirus-essential facilities (PEFs) by National Authorities for Containment (NACs) in consultation with the Global Certification Commission (GCC) have now been established and published, and all countries intending to nominate and certify PEFs should establish NACs as a matter of urgency. Detailed guidelines for establishing and certifying PEFs have been produced by WHO, including milestones and key activities for NACs and PEFs, and all Member States are recommended to become familiar with, and follow, the recommendations provided.

Introduction

Representatives from the WHO European Regional Polio Laboratory Network together with WHO secretariat and international partners participated in the meeting to discuss the status and further development of the polio laboratory network, the poliovirus (PV) containment initiative, and new tools to address laboratory and containment challenges and issues. The full list of participants is provided in Annex 1.

Dr Eugene Gavrilin, coordinator of the Regional Polio Laboratory Network, opened the meeting on behalf of the WHO Regional office and presented the meeting's programme, goals and tasks. Dr Javier Martin was chairman of the meeting; Dr Ray Sanders was rapporteur.

Day 1. Laboratory Network

Session 1. WHO polio laboratory network - global and regional updates

Overview of the performance of the GPEI and Global Polio Laboratory Network (GPLN)

Dr Ousmane Diop provided a summary of the current status of GPEI activities and challenges. As of 22 August 2017, only 20 wild poliovirus (WPV) cases had been detected globally in the past 12 months, 9 in Pakistan and 11 in Afghanistan. It is of concern that WPV was detected in 2016 in northern Nigeria after almost 2 years without detection, and WPV-positive environmental samples have been detected in parts of Afghanistan and Pakistan with no reported polio cases, raising the possibility of ongoing 'silent' transmission in some areas. Detection of WPV remains a mandated declarable Public Health Emergency of International Concern under the terms of the International Health Regulations. Over the same time-period, a total of 42 circulating vaccine-derived poliovirus

type 2 (cVDPV2) associated cases were detected, 1 in Nigeria, 7 in the Democratic Republic of Congo, 1 in Pakistan and 33 in Syria. On a positive note, it is now almost 5 years since the last detected WPV type 3 (WPV3) case and positive environmental sample were reported, suggesting that WPV3 has joined WPV type 2 (WPV2) in the list of globally eradicated pathogens.

In Afghanistan and Pakistan WPV type 1 (WPV1) has been detected in 9 remaining transmission clusters, with the number of WPV1 cases in Pakistan showing a significant decrease in 2017, but the number of positive environmental samples showing no decline. There has been persistent recurrent WPV1 isolation from environmental samples collected in Quetta, Peshawar and Karachi. In Afghanistan, recent cases were linked to cross-border transmission from Pakistan, with subsequent local circulation. However, there has been a decline in the number of positive environmental samples in Afghanistan in 2017. Significant challenges to the programme in Afghanistan and Pakistan include the remaining inaccessible areas in the Eastern and Southern parts of the region, the high risk mobile populations that traverse the area, and the limitations placed on direct field monitoring of activities by senior polio staff.

In July and August 2016, a total of 4 WPV1-associated cases were detected in north-eastern Nigeria in under-immunized children from conflict-affected areas. Genomic sequencing of the isolates determined that the viruses were related to previously endemic strains and that transmission had been occurring for between 2 to 5 years in the absence of detected cases. Two cVDPV2 isolates were also detected in Borno and Sokoto states in 2016, together with a VDPV2-positive environmental sample. Surveillance indicators for the area, previously believed to be strong, were shown to include systematic errors in data collection and assessment, with an almost complete lack of information from inaccessible districts. The regional response to WPV1 and cVDPV2 detection was multiple rounds of vaccination, beginning with supplementary immunization (SIA) rounds in large areas of Nigeria, Cameroon, Niger and Chad using trivalent oral polio vaccine (tOPV) from August to December 2016, followed by SIA rounds using monovalent polio vaccine type 2 (mOPV2). These rounds targeted 40 million children.

Response to the cVDPV outbreaks in the Democratic Republic of Congo and Syria has been extensive SIAs using mOPV2. There have, however, been severe logistical challenges to delivery of vaccination campaigns in both countries. In Syria 33 cases have been detected to date with a median age of 16 months.

In rising to the challenges presented by the final phases of global polio eradication the GPLN has consistently evolved to be faster, more focussed and fully accountable. Target times for PV detection and response to WPV outbreaks has been reduced through introduction of new testing algorithms, increasing capacity for intratypic differentiation (ITD) and by arranging faster shipment of isolates for genomic sequencing. The Network has become more focussed through implementation of comprehensive quality assurance procedures at all levels, provision of enhanced technical assistance to laboratories where needed and establishment of extensive environmental surveillance systems. Network accountability has been increased through systematic improvement of all aspects of laboratory management and introduction of real time laboratory performance tracking. Of the 146 laboratories in the GPLN, 47 conduct virus isolation alone, 73 conduct virus isolation and ITD, and 26 conduct virus isolation, ITD and genomic sequencing. It is likely that the number of laboratories conducting ITD and genomic sequencing will further increase prior to global certification.

As the trend towards a declining number of WPV cases since 1998 has progressed, there has been a general stepwise increase in the number of samples from AFP cases processed in GPLN laboratories. In 2016 in excess of 245,000 stool samples from AFP cases, contacts or other sources were processed in GLPN laboratories. Of these the vast majority had PV isolation results reported within 14 days or receipt and greater than 90% had ITD and/or sequencing results reported within 7 days of isolate receipt.

Development and implementation of environmental surveillance for polio has been a major innovation in recent years and the GPLN is currently working on standardized monitoring indicators to track the effectiveness of environmental surveillance and provide guidance and recommendations on the management, analysis and reporting of environmental surveillance data. Despite the challenges there has been a significant incremental increase in the number of environmental samples collected and processed since 2014, particularly in the WHO African, Eastern Mediterranean and South East Asian Regions. A quality assurance programme for environmental surveillance is in development with a checklist for accreditation to be pilot tested and a proficiency testing scheme to be introduced.

The main threats to GPLN performance include the increasing workload faced by the laboratory coordinators which is having a negative impact on laboratory performance by decreasing provision of routine monitoring and guidance. There is also the threat of complacency setting in as the final stages of global polio eradication approach. There have been an increasing number of non-coordinated demands on the GPLN from the GPEI and National authorities seeking laboratory information required to mount specific programmatic responses to events. An additional challenge facing the GPLN lies in establishing a strategy for maintaining high-quality laboratory-based surveillance for PV for a period beyond global cessation of virus transmission and global certification of eradication. Available funding for the GPLN in its current format will not be available, and surveillance activities will be required to transition into some other format in order to maintain a polio core capacity and sustain polio eradication. More discussion is required at all levels as to how this will be achieved. This was a key area for discussion during the 23rd Informal Consultation of the WHO GPLN was in Geneva in March 2017. At this meeting, it was agreed that the changing GPEI landscape necessitates greater exchange between the laboratories and the programme, particularly with surveillance staff, and that polio assets will either need to be taken over by national authorities or polio surveillance integrated into broader disease surveillance systems. There is an urgent need to align and consolidate the GPEI partners' visions of the future of the GPLN, and questions over the transition process need to be answered sooner rather than later.

Overview of the performance of the GPLN in Europe

Dr Eugene Gavrilin provided a summary of recent key achievements, activities and challenges in the European Regional Polio Laboratory Network. Laboratories in the Region, including Network and non-network facilities, currently process approximately 100,000 samples per annum, with an average weekly workload across the network of approximately 250 samples. There are currently 47 polio laboratories in the European Polio Laboratory Network and all of them are fully accredited for 2016/17.

Poliovirus surveillance in the Region is achieved through AFP, enterovirus and environmental surveillance, with all Member States using at least one of these methods. Laboratory results for

these surveillance systems are reported through the polio Laboratory Data Management System (LDMS). It is of programmatic concern that despite requests made, neither the United Kingdom nor France provides routine reporting of laboratory results through the LDMS and continue to provide results through *ad hoc* reporting systems.

The vast majority of viruses isolated through PV surveillance are non-polio enteroviruses (NPEV) and until mid-2016 the majority of PV isolates detected were Sabin-like, with the occasional VDPV isolate detected. While positive isolates come from all three surveillance systems, environmental surveillance provides the highest proportion of polio-positive samples. The polio positivity rate from environmental samples is extremely varied, however, with a multitude of confounding factors, including a site selection, sample collection and catchment population, which can enhance or reduce proportional positivity. A significant proportion of reported samples collected for enterovirus and environmental surveillance purposes are not tested in Network laboratories.

There has been close monitoring for SL2 isolates since the tOPV withdrawal took place in April 2016, and following a sharp decline in detections during the first few weeks following the switch to bOPV, few isolates have been detected. Isolation of VDPVs has continued, with isolates coming not only from samples collected in the European Region but also from samples from selected sites in the Eastern Mediterranean Region, including Syria. The majority of VDPV isolates originate from chronic excretors, mainly from individuals known to be suffering from primary immunodeficiency disorders.

At present, 13 Member States have provided notification that they intend to establish PEFs, 10 of these are members of the European Union. Not all Member States that have signalled their intention to establish PEFs have decided on the number of facilities they will require but the provisional Regional total is 39. It is possible that this number will increase due to commercial interests and future vaccine manufacture.

In April 2017, there was a containment breach in a vaccine production facility in the Netherlands that resulted in the release of WPV2. Two operators were exposed to WPV2 at the time of the containment breach and one of these was infected. Both operators were closely monitored for infection and environmental surveillance around the homes of the operators was established. The infected individual stopped excreting poliovirus by the end of April 2017 and environmental surveillance ceased after the first week of May 2017.

All 47 laboratories in the network have now switched to the revised laboratory diagnostic algorithm for PV isolation, providing results in a shorter timeframe than the previous algorithm in use. However, this has also resulted in a reduction in the non-polio enterovirus (NPEV) detection rate in network laboratories. The next step is to prepare the network for direct PV detection without the requirement for cell culture, making laboratory containment easier to manage in the long term. In preparation for this, plans are in development to enable every laboratory in the network with the capacity to conduct PV detection and identification using PCR.

Update on the conclusions of the European Regional Certification Commission for Poliomyelitis Eradication (RCC) in 2017

Dr Sergei Deshevoi provided an update on the conclusions and recommendations from the 31st meeting of the RCC held in Copenhagen from 31 May to 1 June 2017. The RCC, expressing

concern over continuing immunity gaps and evidence for continuing 'silent' transmission around remaining endemic foci, urged all Member States, including those in the European Region, to reduce remaining immunity gaps in underserved populations and maintain vigilance for evidence of transmission of VDPV and WPV.

In line with the move towards collecting and collating evidence required for global certification, the RCC has progressively adopted an approach to evaluation of annual update reports based on risk-assessment and evidence of risk mitigation. A more stringent application of the risk-assessment approach has resulted in an increase in perceived risk in a number of Member States that had previously been considered at low or intermediate risk. It has also resulted in a distinction being drawn between Member States that are at high risk due to programmatic failure and those considered to be at a potential risk due to administrative failure. Programmatic failures include failure to establish adequate population immunity; failure to establish or maintain adequate poliovirus surveillance; or failure to respond adequately to a previous outbreak of vaccine preventable disease. Administrative failures include failure to provide the RCC with adequately documented evidence of high population immunity, high quality poliovirus surveillance or successful control of previous events, outbreaks or challenges.

While annual reports were received from each of the 53 Member States in advance of the start of the meeting, only 24 reports were received before the agreed deadline of 15 April 2017, while 10 were received after 15 May 2017. Some of the reports received, especially those received immediately before the meeting, were superficial and lacking in detail. And the RCC urged all national certification committees (NCCs) to make efforts to provide the WHO Secretariat with full and detailed reports in advance of the agreed deadline for submission.

Based on the evidence provided, the RCC concluded there was no WPV transmission in the WHO European Region in 2016. However, Bosnia and Herzegovina, Romania and Ukraine remain at high risk of a polio outbreak following importation, due primarily to low population immunity. Twenty-five countries were considered to be at intermediate risk, and no assessment could be made for Italy since no NCC has been established and the RCC was unable to issue a formal risk assessment in the absence of an NCC.

The RCC expressed satisfaction on the success with which the polio outbreak simulation exercise (POSE) package and experience has been developed and deployed, and the interest now being shown in POSE by other WHO Regions. Every Member State should be conducting frequent POSE activities and POSE should now be tailored specifically to focus on the greatest risk faced by the country.

The RCC expressed concern that due to significant delays in the global provision of IPV five Member States in the Region (Republic of Moldova, Kyrgyzstan, Tajikistan, Turkmenistan, and Uzbekistan) have been unable to introduce a single dose of IPV to supplement their introduction of bOPV. These countries are currently not providing their populations with protection against PV type 2. The RCC urged the global programme to give a higher priority for supply of IPV to these countries as it becomes available through 2017 and 2018.

With regard to PV containment the RCC recommended that all countries considering the establishment of PEFs make themselves fully aware of the international requirements, including

maintenance of an effective national routine childhood polio immunization programme and high national population coverage with polio vaccine, and the exacting and time-consuming PEF containment certification requirements.

Discussion

The RCC also expressed concern that a significant number of Member States are not meeting the agreed requirements against recommended surveillance standards and are urged to improve surveillance quality and provide full surveillance documentation in the requested format. Information on enterovirus and environmental surveillance systems and results, including the origin of samples tested and virus isolation rate, is lacking for several countries. Countries have been requested to provide more data on their enterovirus end environmental surveillance results, particularly where AFP surveillance is absent or poorly performing.

The RCC also requested Member States to ensure that all non-typed enteroviruses isolated from patients with polio-compatible clinical conditions are screened to exclude PV. This request applies to samples tested in non-network laboratories. All PVs detected must be forwarded to an accredited WHO polio laboratory for intratypic differentiation and any further characterisation required.

The current polio LDMS can be used to record and report laboratory results on enterovirus and environmental surveillance samples, but in general the level of data integration between laboratory and field epidemiology for these supplementary surveillance programmes is not high. Further discussion and development is needed to improve the quality of reporting supplementary surveillance results to the programme and systematising the analysis and response to positive results. The LDMS can also potentially be extended for use as a platform for non-polio enterovirus surveillance, but this requires further discussion and development.

Session 2. External quality assurance - proficiency testing (PT)

Isolation PT update

Dr Erwin Duizer provided a summary of the 2016 isolation PT panel and results. The 10-sample panel included 8 positives and 2 negatives, with 6 single virus samples and 2 mixtures. Six of the samples contained poliovirus (all SL) and 4 contained NPEV. Two of the PV samples were at low titre. The maximum score for the test was 100% and a passing score was ≥90%. Twenty percent was deducted for a false positive or false negative L20B culture, resulting in a failed test. Five percentage points were deducted for other failures. Twenty laboratories in the European Region scored 100%; 10 scored 95%; 2 scored 90%; and 4 scored ≤80% on the first attempt. Of these 4, all scored ≥95% on retest.

The test panel for the 2017 PT is currently being validated, and will contain slightly higher titres of PV, than the 2016 panel. There will be no SL2 in the panel and distinct SL types 1 and 3 will be used in preference to vaccine virus. Shipping of the panels directly from the PT coordinating facility at RIVM to the national laboratories remains a challenge for some WHO Regions. The pro-forma invoices are currently being prepared for sending in early September and shipping of the panel will start as soon as possible after that.

ITD PT update - PCR and sequencing

Dr Steve Oberste presented the PT results for PVITD and genomic sequencing. Composition of the 2016 ITD PT reflected universal use of the ITD 5.0 algorithm and included 10 samples, including a range of single PVs, mixtures, Sabin-like and non-Sabin-like viruses and viruses expected to give discordant or indeterminate results. The maximum score was 100% and the minimum passing score was 90%, with 15% deducted for failure to detect or identify WPV, VDPV or any PV type 2. Deductions were also made for failing to detect a single Sabin virus or detect a Sabin virus in a mixture. Deductions were also made for a number of technical and administrative failings including failure to follow the testing algorithm, not recognising a failed control, incorrect interpretation of results, late reporting and not being ready to process the panel on arrival in the laboratory.

Of the 11 laboratories in the European Region taking the test, 2 scored 100%; 3 scored 95% (failing to identify atypical WPV1 signals); 2 scored 90% (failing to identify strong positive-like WPV1 signals); and 4 laboratories scored <90% on first testing (multiple mistakes in both testing and reporting). The majority of problems detected appeared to relate to training issues and some relate to the different machines used by laboratories in the Region. This is a problem for the European Region in particular where there is wide heterogeneity in the range of machine in use in different laboratories presenting a challenge for standardization. Additional training workshops have been planned, particularly for laboratories facing difficulties. The 2017 ITD PT panels have been prepared and will be distributed in September or October.

The 2015-2016 sequencing PT panel consisted of 4 lyophilized non-infectious PV RNA samples. Three of the samples consisted of individual virus RNAs and 1 sample consisted of a mixture of Sabin viruses. The primers for use in the 2016 PT were shipped to all participating laboratories with the 2012 PT samples. Laboratories were requested to provide complete documentation on their results, including gel images, raw data, edited contigs and the final consensus sequences. An additional sample, provided on an FTA card, was included as a pilot test of the card use and results were not scored. Of the 8 laboratories from the European Region participating, 4 scored 100%; 1 scored 99%; 1 scored 95% and 2 scored <75%. Of these two, 1 scored 100% on retesting. Globally 20/25 laboratories achieved scores of ≥90%. In all, 20/25 laboratories reported results and files for the FTA card with high quality, but several were challenged in acquiring the materials needed for FTA card processing and the RNA processing buffer (glycogen and DTT).

Problems observed with the test included weak bands obtained with one of the samples, low quality raw data and a failure to report complete VP1 sequences. These were also a number of problems with interpretation of results and documentation. Many of the issues can be resolved through further training.

The 2017 sequencing PT will consist of 6 samples, 3 of which will be provided as lyophilized control RNAs and 3 as samples on FTA cards. Laboratories will be required to submit complete sequencing results within 7 days of receiving the panel and a score of ≥90% will be required to pass. Distribution of panels began in July.

Discussion

All participating laboratories are requested to provide feedback on their experiences with the sequencing PT, the challenges they encountered and any comments and suggestions on how the PT panel and process can be improved.

Session 3. Developing strategies for the future

A way of life post-certification: WHO/Europe experience

The WHO European Region was certified polio free in 2002, and Dr Eugene Gavrilin discussed the primary laboratory challenges in demonstrating a continued polio-free status and the potential for developing future polio surveillance systems. A major challenge has been that AFP surveillance appears to be the only standardized high specificity system that is comparable across all countries. However, several countries in the Region have never established AFP surveillance, and of those that have, some have found it difficult to maintain systems that meet the surveillance quality criteria. Enterovirus surveillance is used as an alternative or supplementary surveillance system in many countries, but this has proven to be very challenging to evaluate, tends to lack specificity for polio and in most cases, has little to offer over random stool surveys. Environmental surveillance is a challenge to establish, standardize, monitor and achieve sufficient large-scale coverage of the population to meet surveillance sensitivity criteria. Standard laboratory PV detection methods also make use of cell culture, which carries an inherent risk in a polio-free environment and will require strict laboratory control in a polio-free world. Direct PV detection in samples would overcome the need for culture-based detection, but current methods pose particular problems for some specimen types.

A significant impediment to consideration of future PV detection systems has been the assumption that there must be zero missed PV infections in future scenarios. While risks can be minimized, practical considerations determine that zero risk is not achievable in any real sense, and future polio surveillance systems must accommodate this concept. Defining the level of acceptable risk requires discussion and further research, but moving away from the requirement for 100% sensitivity will aid development of new systems. Future systems are likely to make use of convergent technologies, including biosensors and internet-based applications, and may well move away from specific pathogen detection to monitoring of host responses to infection. Systems will need to be scalable, integrated into broader systems and arrays, and probably commercially based.

Laboratory surveillance prospects during the endgame. Laboratory investigation for PVs without virus isolation: progress on direct detection of PV in stool samples using CDC rRT-PCR assays

Dr Steve Oberste provided a discussion on progress made in developing direct PV detection assays in a range of sample types. Diagnostic innovations over last 20 years have already reduced turnaround time for PV detection and characterization by half, with the introduction of a revised isolation testing algorithm, the introduction of ITD 5.0 and standard genomic sequencing methodologies.

As we move through the polio endgame strategy towards global polio eradication questions arise as to whether the virus isolation step can be removed to minimise risk, and whether the laboratory testing time can be further reduced. One potential answer is to make use of direct detection of PV in samples. A great deal of development has been carried out on methods for direct PV detection in stool and sewage, including application in Israel in 2013. Direct detection potentially saves up to 7 days in the laboratory, but direct detection requires much more sample processing and current assays are at the sensitivity limits of PCR. At present sequencing methods are less sensitive than rRT-

PCR by 100- or 1000-fold, raising the question of how to get quality sequence data directly from stool.

Sensitivity limitations may be overcome by adding more material for each PCR reaction (however, that would risk carryover of additional PCR inhibitors) or simply increasing the amount of stool extracted. Studies have suggested that increasing the concentration of stool suspension for RNA extraction from 10% to 50% has limited effect on final RNA copy number obtained. Additional studies have demonstrated that not all RNA extraction kits are equally efficient, but further work is required to determine the most consistently effective kit and the best way to use it. There is an added complication in that there is usually an insufficient amount of virus per unit volume in sewage to detect without a concentration step. Studied have been started on methodologies to enrich or concentrate samples to increase sensitivity, including use of polyclonal antisera, monoclonal antisera and magnetic bead technology. Use of polyclonal antibody has demonstrated little advantage, but use of monoclonal antibodies has shown some promise and requires further investigation. Use of magnetic bead technology has shown good initial results but there are a number of logistical and technical challenges and further investigation is underway.

Adopting direct detection methodologies will have higher costs than currently methodologies, and present challenges with regard to issue and sustainable supply of reagents. Furthermore, direct detection methods must be implementable in existing GPLN labs, especially those that serve high-risk areas, and be implementable in laboratories with no molecular biology experience. These challenges will need to be met before direct detection becomes a commonplace methodology in the GLPN.

Update on RotorGene/Biorad/Stratagene ITD 5.0 validation

An update on the validation of different PCR platforms used for ITD 5.0 was provided by Dr Steve Oberste. ITD assays developed by CDC Atlanta, including ITD 5.0, have been developed and tested using the ABI 7500 and 7500 fast platforms. This platform has now been in use for some time but is not universally available, so there is a need to adapt the assay to other platforms in common use. The challenges to validating use on other platforms include the different heating and cooling technologies in use and the different rates of heating and cooling of reaction mixtures. A study has been undertaken to validate three other platforms for ITD use. These include the Stratagene MX3000/3005, an older machine but widely available; the Bio-Rad Cfx, which uses LED technology, uses fewer moving parts, is low maintenance and is relatively inexpensive, and the Rotor-Gene Q which uses a different heating technology than ABI, does not need calibration and is widely available. All three instruments were tested using a CDC polio isolate panel.

Testing of the Stratagene MX3000 and Bio-Rad Cfx showed 100% concordance with the ABI 7500. ITD 5.0 performance on Rotor-Gene is equivalent to ABI 7500 with 100% agreement between the two machines. However, false negative (panPV) signals were seen that were due to the high background. This problem appears to be not common, and a 1:10 dilution of test sample and retest may occasionally necessary. It is clear that for panPV a proper data analysis is crucial. One possible disadvantage of the Rotor-Gene Q is that the operation is completely different from ABI 7500, Bio-Rad Cfx, and Stratagene MX3000, but the machine is easy to set up. Additional instruments are becoming available and plans are in hand to conduct similar validation exercises on them.

ITD expansion plans for the Region

Dr Eugene Gavrilin presented an account of current plans the implementation status for expansion of ITD capacity in the Region. A survey on the PCR platforms in use in the Region has revealed a high level of heterogeneity of platforms which complicates expansion of ITD capacity using existing assets. Taking this heterogeneity into account an ITD expansion plan developed with technical consultation from WHO HQ and US CDC. Regional laboratories have been grouped according to PCR platform type with the aim of providing platform-specific ITD training workshops. The first such workshop, for laboratories using the Stratagene platform was held in December 2016 at the laboratory in Helsinki. A workshop for the ABI platform was held in January 2017 at the Tel Aviv laboratory. In addition, inter-regional genomic sequencing workshops were held in October 2016 at the Paris and Rome laboratories, and an on-site sequencing workshop was held at the Ankara laboratory in July 2017. Now that the assay validation study for the Rotorgene-Q platform has been completed, a workshop on use of this platform will be held in the near future. All laboratories that have implemented ITD PCR have been requested to inform WHO so that they can be included in the proficiency testing scheme.

Session 4. Investigating samples of programmatic importance

Lessons learned in Turkey from the increase in workload associated with the Syrian cVDPV2 outbreak

Dr Gulay Korukluoglu, head of the Ankara laboratory, described the increase in workload associated with the testing of samples from Syria and the challenges associated with processing a rapid increase in sample numbers associated with the outbreak. The laboratory initially feared an overloading of capacity the process the increased number of samples and conducted a SWOT analysis to review the strengths, weaknesses, possible opportunities and potential threats faced by the laboratory. The assessment revealed a need to increase the laboratory capacity for conducting ITD assays and to develop genomic sequencing capacity. These developments would streamline the overall testing process and greatly reduce the total laboratory testing time. It was also decided to renovate the laboratory to provide greater space allocation for the additional testing and greater workload. The Ankara laboratory now handles more samples from Syrian than samples from Turkey, and the laboratory at Izmir has taken on responsibility for handling samples from some Turkish provinces that would formerly have gone to Ankara.

Day 2. PV. Challenges of containment breach and risk assessment

Session 1. Global and Regional updates on PV containment progress

Timeline, current global status, Containment Certification Scheme (CCS), coordination between PEF/NAC/GCC

Dr Jacqueline Fournier-Caruana provided a presentation on the current implementation status of laboratory containment of PVs, regulatory structures and ongoing activities from the global perspective. PV laboratory containment remains critical to maintaining polio eradication. It requires a complex global programme that involves all 194 WHO Member States together with the 21 non-member countries and territories and has an extended time horizon. The WHO Global Action Plan to

minimize poliovirus facility-associated risk after type-specific eradication of wild polioviruses and sequential cessation of oral polio vaccine use (GAPIII) seeks to provide a modern, comprehensive, risk-based and practical framework to ensure organizations that will handle and/or store stocks of PV after type-specific eradication will do so with due regard for biorisk management (BRM). As long as PV is retained anywhere, containment will be required.

GAPIII requires that all PV type 2-infectious and potentially infectious materials (PIM) be either contained or destroyed following global withdrawal of tOPV in April/May 2016. The PV type 2 containment period, or Phase II of the GAPIII implementation plan, was scheduled to extend from 3 months after the point of tOPV withdrawal to the point of global certification of WPV eradication. There have been delays in completion of Phase I activities in many countries, but most Member States are now either implementing, or preparing to implement Phase II activities. Countries are required to either destroy all WPV2 and Sabin type 2 infectious and PIM, or transfer them to a designated PEF. Details of the GAPIII CCS have now been published. The PEF certification process is conducted within the countries by a NACs. Most countries with PEFs have already established fully functional NACs, others have functional NACs that lack final government approval. There remain three countries in the European Region with nominated PEFs that have yet to establish NACs.

An oversight structure for containment was developed by WHO and in 2016, the GCC fully endorsed the proposed structure, including the establishment of a GCC Containment Working Group (GCC-CWG), to be led by a GCC member. The GCC-CWG, established in December 2016, will start functioning as soon as NACs submit reviews of PEFs applications. In view of required technical expertise for Phase II and anticipated workload, the GCC agreed to delegate day-to-day responsibility for Phase II implementation to the GCC-CWG. There will be a meeting between the chair of the GCC-CWG and representatives of the NACs from IPV-producing countries in October 2017.

A new advisory body, the Containment Advisory Group (CAG), has been established to provide technical advice on issues related to GAPIII implementation. This group held its first meeting in Geneva in June and the report of that meeting has been published. With the publication of the deliberations from the first CAG meeting, and the resolution of a number of technical questions that were seen to be delaying implementation of Phase II, it is hoped that containment activities can now be accelerated. It is expected that there will be a World Health Assembly (WHA) resolution on PV containment discussed at the next WHA meeting.

Regional status of PV containment activities - European Region

An overview of PV containment implementation in the Region was provided by Dr Maria Iakovenko. In total 38 Member States in the Region have declared they have retained no WPV2 or Sabin type 2-related infectious materials. Two Member States have declared they have Sabin type 2-related infectious materials but plan to destroy them. Thirteen Member States have declared they have retained WPV2 and/or Sabin type 2-related infectious materials; of these 1 will establish PEF for Sabin-related materials and 12 will establish PEFs for WPV and Sabin-related materials. Of the 13 Member States that have declared their intention of establishing a PEF, 8 have established a NAC; 2 have an acting NAC; and 3 have not yet established a NAC. The European Region faces a challenge in that the majority of poliovirus vaccine production facilities are located within the Region. Of the 13 Member States intending to establish PEFs, 6 of them host polio vaccine manufacturing facilities on

a total of separate 12 sites. BRM training for proposed PEFs and NACs was provided in 2016 and the training for auditors for CCS was conducted in 2017 with altogether more than 70 people were trained to ensure the appropriate expertize in relevant Member States.

Regional status of PV containment activities - Western Pacific Region

Dr Varja Grabovac, from the WHO Regional office for the Western Pacific, provided a presentation on the achievements, progress and challenges of GAPIII implementation in the Region. There are 5 Member States in the Region hosting a total of 16 PEFs. BRM training for proposed PEFs and NACs was provided in 2015 and 2016, with training of auditors for CCS conducted in 2017. The Regional Office is currently working with countries to establish NACs.

Among the challenges encountered include the shortage of financial and human resources available for containment activities and the complexities of identifying and handling Sabin-related type 2 infectious and potential infectious materials in non-polio laboratories. Several of the nominated PEFs are not yet ready for GAPIII and require refurbishment and upgrading to improve biosecurity and biosafety. Member States also struggle with a lack of national legislation to implement the requirements of GAPIII and innovative solutions are being sought by some. NACs have been identified but absence of clear terms of reference and lack of auditor training call their functionality into question. As in other Regions, some members of proposed NACs are experts associated with the PEFs, and as such may have conflicts of interest. NACs in 4 Member States will be called upon later this year to begin the PEF certification process, and further training for auditors and a workshop for NPCCs will be taking place.

Laboratory performance strategies in the GPLN in the context of PV containment

Dr Ousmane Diop described the development and milestones of the strategy for BRM and PV containment within the GPLN. The WHO BRM programme began in 2010 in response to an observed lack of systematic biosafety and biosecurity systems in many facilities and institutions hosting GPLN laboratories. Phase 1 of the BRM programme consisted of awareness raising and preparation and distribution of a DVD package on biosafety and biosecurity. Phase 2 consisted of the programme established the concept in all WHO Regions and established training of trainers' courses of laboratory staff and coordinators. At this point the programme was considered only partially successful due to low levels of implementation in some laboratories. Poor implementation was associated with lack of institutional support in some cases and shortage of adequate tools and commitment in others. Phase 3 of the BRM programme is linked to GAPIII implementation and the linkage has been reinforced through a series of training given through 2015-2017 in each of the WHO Regions. The programme is currently providing support to laboratories to comply with GAPIII Annex-6 by providing an adequate tool for gap assessment and correctly identified gaps or weaknesses.

A series of guidance papers have been issued to support laboratories in meeting GAPIII requirements while maintaining diagnostic capacity. The first describes safe handling and storage of PV2 in GPLN laboratories. The second provides an update on the ITD molecular assay and testing algorithm, while the third provides guidance on the polio-specific antibody testing of GPLN personnel.

Potentially infectious materials (PIM) - progress in the development of Guidance on completion of Phase I: concept approach

Dr Nicoletta Previsani provided a presentation on the status of implementation of the first phase of PV2 containment and current thinking on risk associated with PV PIM. Although Phase Ia of PV2 containment has been completed in many Member States the use of mOPV2 in response to cVDPV2 outbreaks and resistance to destruction of PV2 reference-materials in some non-polio-network laboratories has delayed implementation in some Regions. Guidance has been prepared for non-poliovirus facilities to minimize the risk of sample collections of PIMs with the aim of assisting them to assess the risk of PIMs in their possession and implement appropriate risk reduction consistent with GAPIII. The guidelines describe 3 levels of risk associated with PIMs and provide appropriate risk mitigation strategies. The guidelines document is currently being finalized, to include the most recent recommendations from the CAG, and a draft will soon be posted on the web for public comment and pilot testing. The final draft will be submitted to the CAG for endorsement in November and it is hoped the final document will be published before the end of 2017.

Discussion

It would be helpful to present the containment requirements for diagnostic laboratories and PEFs in a simple and straightforward manner, such as an algorithm that NPCCs and NACs could follow. Some of the work has already been done in the Region and a series of background documents and resources are already available, and further documentation and material is in preparation.

An assessment tool for cost analysis of establishing PEFs is in the final stages of completion and will be distributed for comments after the meeting.

Session 2. Sharing experience and progress on PV containment implementation

Handling and holding of PV2 isolates in non-PEFs and compliance with containment requirements, strategies and issues

<u>Experience from Israel</u>

Dr Merav Weil provided a presentation on the activities and challenges faced by the Israel National polio laboratory. The laboratory routinely receives and processes samples from AFP cases, enterovirus surveillance, newly diagnosed primary immunodeficiency (PID) cases and environmental surveillance from Israel and areas of the Palestinian Authority. The laboratory will also soon be receiving samples from Syrian patients, who received humanitarian treatment in Israel, and their accompanying persons because of current circulation of cVDPV2 and the mOPV2 vaccination campaign. Most of the samples received have a low risk for PV2, but the risk of cVDP2 remains and virus can still be imported into Israel of areas of the Palestinian Authority. In addition, PID patients have been demonstrated to be capable of excreting PV2 for prolonged periods and PV2-positive PID patients are considered to be 'hot cases'. Isolates from these cases are rapidly sequenced to obtain evidence for potential virus transmission and to determine an appropriate response.

The laboratory is not a PEF, but all potentially PV2 infected samples and samples from 'hot cases' are subjected to thorough risk assessment and handled and processed appropriately. Samples

considered to present a risk are separated from routine samples and handled in the onsite Biological Safety Level 3 (BSL-3) facility according to strict BSL-3 Standard Operating Procedures (SOPs). All working materials are stored at -70^{o}C within the BSL-3 facility and all materials for discard are autoclaved before removal from the facility. RNA extraction for ITD and sequencing is conducted within a closed Biological Safety Cabinet. After analysis all materials are destroyed, except for the original samples from special cases which are securely stored in a restricted area and sent to the PEF for further research.

The risk assessment process is based on the facility location, personnel, infrastructure, and available equipment, and must be frequently reviewed and updated by certified safety officers especially when conditions change. Changing conditions may include elevated or reduced risk for finding PV2; availability of new advanced equipment; changes in infrastructure of the facility; and changes in detection methods available. Direct detection of virus from the original sample would significantly reduce the risk of accidental virus spread.

Experience from Turkey

Dr Gulay Korukluoglu provided a presentation on the experience gained in the Turkey National polio laboratory. The laboratory receives routine samples from Turkey and samples from Syria, including samples collected during the Syrian cVDPV2 outbreak. The laboratory was accredited to ISO 15189 standard in 2016 but requirements for maintenance and monitoring of systems, including staffing, equipment and funding, remain a challenge. Following a risk assessment, the laboratory facilities were upgraded to include a controlled access and an enlarged negative pressure area and renovation or replacement of laboratory equipment. Experience has shown that the most significant challenges to efficient and safe function include the time required for detailed inventory of all materials together with full records of all processing and detailed reports of all findings. This level of increased documentation is essential for biosecurity, but is resource- and time-intensive. There are also concerns over the increased biosecurity required for the transport of potential PV2-infectious materials, particularly those coming from Syria. Safe transport of these samples is expensive.

Verification of destruction of PV2 materials in accordance with GAPIII requirements

Dr Zalimkhan Omariev provided a presentation on the experience of validation gained in Russia. A centralised, hierarchical system has been established by the national authorities of coordination of all safety concerns related to International Health Regulations. The system includes extensive state laboratories throughout the country together with mobile teams for investigation of events and outbreak response. There is a coordinated policy of control that includes rules and guidelines on sanitary control. A National Plan was developed in 2015 that includes the requirement for eventual destruction of all poliovirus-infectious materials outside of designated facilities. There are currently 6 facilities identified for the safe holding of Sabin type 2 materials and 1 facility for the holding of WPV-infectious materials. Currently more than 90% of all laboratories likely to be holding PIMs are within the centralised system.

There is a process for documented destruction of all unwanted PV-associated materials, based on established national guidelines and supported by legislation. A national commission is responsible for the auditing of laboratories holding PV and PIMs, and the audit reports have been used to prepare a list of potential PEFs. The audit commission has also maintained lists of laboratories that

have already documented destruction of PV and PIMs. GAPIII requirements have been documented as present in all laboratories.

Setting up a biosecurity system the Danish experience

Dr John-Erik Stig Hansen provided a discussion on the requirements for establishing a national biosecurity system, based on experience gained in Denmark. As biosecurity represents an important element in the CCS and overall biorisk management system, countries may find this experience useful. Assessment of biosecurity arrangements in Denmark in 2006 exposed potential weaknesses in several major facilities in the country and resulted in establishment of a national programme to strengthen biosecurity. An act of Parliament passed in 2008 and Executive orders in 2009 supported this process. A licencing process has been established to limit the number of facilities able to work with controlled agents, but balancing against the need to maintain laboratory research and development. Stringent biosecurity requirements must be met before a facility is licenced, and licences can be withdrawn if the terms of the licence are infringed. The process also allows for custodial sentences to be handed down in case of gross infringement. After 7 years of licencing, approximately 160 licences have been granted to laboratories working with controlled agents, industrial companies, diagnostic laboratories in clinical microbiology and selected retailers of reference agents and materials. Much of the time of the agency is spent on awareness raising, education and promotion of the requirements, and inspection and assessment of facilities. A practical guidance on establishing a national biosecurity system published by the Danish Center for Biosecurity and Biopreparedness in English and Russian is available on the Center's website.

Establishment of the NAC and engagement of PEF into certification process

Dr Katherina Zakikhany provided a presentation on the establishment of a NAC and engagement of a PEF in Sweden. OPV was never used in Sweden, and the last detected case of polio occurred in 1977. The national polio laboratory, located within the Public Health Agency of Sweden (PHAS), is not a PEF, but a vaccine manufacturer in Stockholm has been nominated as a PEF. For this reason, a NAC is required under the requirements of GAPIII, but it was not immediately obvious which national body had the authority to establish a NAC. Despite the absence of a national or EU legal framework, it was eventually decided that the PHAS had sufficient mandate to establish a NAC and had sufficient expertise in the area of biosafety and biosecurity. A NAC, consisting of members with appropriate expertise and professional qualifications, was then established.

The vaccine producer is fully compliant and willing to engage in the PEF certification process and 2 meetings between the NAC and the producer have taken place to discuss certification strategy. The proposed PEF has performed a GAP-analysis based on GAPIII Annex 3 and managers have expressed a desire to participate in WHO GAPIII training workshops. The application for a Certificate of Participation (CP) is being prepared and will be submitted shortly.

The 2 main challenges faced are the recruitment of suitable auditors when available human resources are limited, and establishing a certification process for a facility that is a fully active vaccine production site and stoppages to production must be avoided as much as possible.

Preparation of a PEF for certification compliance with GAPIII Requirements

Dr Javier Martin provided a presentation on the activities undertaken in the UK to prepare the National Institute for Biological Standards and Controls (NIBSC) for GAPIII compliance. The UK initially conducted work on a strategy for GAPIII implementation and established a NAC under guidance from the national Health and Safety Executive. NIBSC was proposed as a PEF due to its work related to vaccine development and quality control and the preparation and storage of WHO International Biological Standards. NIBSC also serves as a global repository for WPV and VDPV isolates of interest. The main challenge to developing the implementation plan has been to locate appropriate experts within the UK that are not directly associated with the PEF and so have no potential conflicts of interest. The CP is currently being developed and full engagement of the PEF is ongoing. The process of certifying the PEF is expected to take approximately 1 year.

A number of legal and technical questions remain, including potential conflicts between GAPIII requirements and the UK legal framework on dangerous pathogens, containment requirements for handling samples from long-term chronic PV excretors, the physical location of long-term storage facilities for PV infectious materials and the containment requirements for continued use of PV2 in standard serological assays.

Discussion

Concerns have been raised over the requirement to demonstrate that PV RNA has been inactivated before it can be safely removed from the containment area. Heat inactivation has been demonstrated to be effective while retaining capacity to provide complete VP1 sequences from inactivated material. A method is currently being validated and the protocol will be made available.

While direct detection of poliovirus shows great promise, detection of specific isolates against the high background of viruses normally present in environmental surveillance samples remains a challenge. It appears that an initial cell culture step will continue be required for some time, increasing the need for thorough risk assessment and bio-vigilance in laboratories. Studies are ongoing on methods for selectively enhancing PV detection in complex mixtures, and results from these will be announced in due course.

Session 3. Containment breach and response procedures

Spill on the IPV manufacturing site, response measures and lessons learned: Netherlands experience

Dr Erwin Duizer provided an account of the recent WPV2 spill at a vaccine production facility in the Netherlands and the responses and follow-up activities undertaken. In April 2017, there was a containment breach in a vaccine production facility in the Netherlands that resulted in release of WPV2. Two operators were exposed to WPV2 at the time of the containment breach. Stool and throat swab samples were collected from both operators and 4 days after the spill it became evident that one of the operators had been infected. The National Response team and WHO were alerted but it was realised that the Public Health Response guidelines had not yet been updated to comply fully with GAPIII containment requirements and that the national polio laboratory was under-prepared to handle PV2-infectious diagnostic materials of this nature. GAPIII Annex 6 was followed as reference for initial laboratory isolation and characterization activities.

Positive isolates from the infected operator were confirmed as WPV2 six days after the spill, and all materials originating from the infected operator were then processed according to GAPIII Annex 2 guidelines within the BSL-3 facility. The infected operator was quarantined at home, in a small city in the centre of the Netherlands situated in reclaimed land. Environmental surveillance at 3 sites around the home of the operator was established, covering a population of approximately 1260 households, and a total of 4 positive samples were detected by PCR and virus isolation. The infected operator stopped shedding WPV2 4 weeks after exposure, slightly after the last positive environmental surveillance sample was collected. In all, 110 stool samples, 12 throat samples and 40 sewage samples were collected, and 6 polio antibody assays were conducted. Together with response team and local public health services activities the total costs are estimated at €200,000.

Lessons learned during this event include the requirement for a new national follow-up protocol following possible exposure that meets the requirements of GAPIII. The protocol should include the requirement for daily stool sampling to begin immediately. Experience has shown that throat swabs are of marginal value, being rarely positive. Similarly, although serology clearly works, the added value is questionable given time frame of results becoming available. Quarantine of the exposed operator in their own home presented significant challenges to the PH response team, including the expense and effort required to establish environmental surveillance around the home. Isolation of potentially exposed individuals in a designated containment environment would be far more effective in terms of ease and safety of investigation and cost effectiveness. The experience has demonstrated the need to update national public health guidelines to meet GAPIII requirements.

Ranking of PEFs the risk assessment approach

Dr Jeff Partridge described work of the WHO Containment Management Group (CMG) in developing a strategy for ranking the risk presented by PEFs under different circumstances and in different environments. GAPIII differentiates between the risk associated with retaining WPV2/VDPV2 (Annex 2) versus retaining OPV2/Sabin2 (Annex 3). The CMG undertook the PEF risk ranking exercise to stimulate discussion on risk at any PEF relative to other PEFs, align risk mitigation priorities with current existing risk, to align risk mitigation priorities with the containment “pre-requisites” to be met by the time of WPV eradication certification, target country-specific advocacy efforts and inform PEF reduction advocacy.

For the purposes of the exercise risk was defined based on increasing probability of infection given exposure and increasing probability of transmission in a community following virus introduction. PEFs were categorized according to the infectious content of materials held (WPV2/VDPV2 vs OPV2/Sabin2), the amount of infectious materials held (vaccine manufacturing vs laboratories), national type 2 vaccination coverage in two strata (< 90% or ≥ 90%) and national percentage access to improved sanitation in two strata (% access ≤ 95% or % access > 95%). On these criteria PEFs were assigned a rank of 1 to 4; rank 1 representing highest risk and rank 4 representing lower risk. The assessment prioritizes WPV2/VDPV2 (as opposed to OPV2/Sabin2), higher infectious content of materials held, lower national population immunity and lower access to improved sanitation as presenting greater risk.

Preliminary tables of risk ranking of proposed PEFs in different countries have been produced, but these rankings are subject to change as countries finalize reports with further details of virus

materials retained and their reasons for retention. The assessment protocol remains in development and countries are requested to provide comments and suggestions to the CMG for consideration.

Day 3. New tools to address laboratory and containment issues

Session 1. New containment inventory and LDMS

Progress in e-Inventory development: concept and demonstration

Dr Maria Iakovenko provided a description and demonstration of the electronic inventory tool being developed by the Regional Office in support of PV containment activities. The database is being developed an a more convenient way for countries to share their PV inventory data with WHO and for the countries and WHO to store all necessary information in an easily searchable format making use of the SharePoint platform. Except for a small number of adjustments required for the user interface the project is in the final stage of completion. The platform will be made available through secure access to all NPCCs and to WHO. Access can also be given to WHO HQ. If other WHO Regions are interested in the platform it can be made available to them for adaptation to their own needs.

New LDMS platform: concept and demonstration

Theo Kaloumenos provided an account of the new LDMS being developed to replace the existing polio LDMS. The new system will be a web-based platform that is integrated with the measles and rubella laboratory data management system. The new system will have co-functionality with the existing polio LDMS, but in addition to being a web-based data entry system, will have a number of additional features, including the capacity for bulk upload of data and improved reporting. English and Russian language editions are being developed. Development is progressing but, as yet, no definite release data has been announced.

Meeting summary

- Available evidence strongly suggests that the global interruption of wild poliovirus (WPV) transmission is imminent, with only 9 WPV cases in the world detected in 2017 as of 28 August, and transmission limited to Afghanistan and Pakistan. However, continued detection of WPV-positive environmental samples in Afghanistan and Pakistan in the absence of detected cases suggests ongoing silent transmission in some areas. Furthermore, the continuing detection of circulating vaccine derived poliovirus (cVDPV) associated cases in the Democratic Republic of Congo and Syria indicates poliovirus (PV) immunity gaps in these countries. All Member States need to continue their efforts to reduce remaining immunity gaps, particularly in underserved populations, and maintain high quality laboratory-based surveillance for evidence of transmission of WPV and VDPV.
- The revised laboratory testing algorithm for PV isolation has been successfully adopted by all laboratories and the new ITD 5.0 algorithm has been adopted by all laboratories conducting ITD. Most Network laboratories are now using the WHO Laboratory Data Management System (LDMS) to report results.
- All laboratories in the Region continue to perform well and have been WHO accredited for the past 3 years. All laboratories passed the 2016 PV isolation proficiency test, although 4 failed at their first attempt. Of the 13 laboratories participating in the 2016 ITD proficiency

tests, four failed to pass the first round due to multiple issues, many related to training issues. Eight laboratories in the Region participated in the genomic sequencing proficiency test. This test continues to evolve, with the 2017 panel including some test samples provided on FTA cards.

- The Regional plan to expand ITD testing capacity to include additional laboratories is in place and is being implemented. Expansion is challenged by the high level of heterogeneity in the PCR platforms currently used in laboratories in the Region. Training in the use of some of the most common platforms has been provided and further training activities are planned. To date, 14 laboratories have implemented ITD 5.0 but only 10 have been accredited for its use.
- Work on developing methodologies for direct PV detection in stool and sewage samples continues and is progressing. As with any other laboratory methodology, some compromise between sensitivity and specificity will probably be necessary. Direct detection methods must rapidly flag samples for genomic sequencing and permit rapid identification of “hot cases”, but complex mixtures of viruses, particularly in environmental samples, remain a challenge for genomic sequencing and direct detection may compound this. Potential methodologies continue to evolve, but further discussions are required on acceptable sensitivity and specificity, and how to interpret and respond to results.
- Work is ongoing on the adaption of the ITD 5.0 to platforms other than the ABI platform on which the test was developed. In addition to ABI the assay has now been validated on machines from BioRad, Stratagene and Rotor-Gene and protocols are available for use on each of these platforms.
- Network laboratories can face unexpected increases in laboratory workload due to outbreaks and emergencies and should all have preparedness plans in place to effectively deal with these potentially disruptive situations.
- Laboratory containment of PV2 is progressing but is behind the planned timeline and there is an urgent need to accelerate the rate of implementation.
- Roles and responsibilities for containment, together with oversight and technical support and advice mechanisms have been established and are now in play, including establishment of the Global Certification Commission Containment Working Group (GCC-CWG) and the Containment Advisory Group (CAG).
- Mechanisms for the certification of poliovirus-essential facilities (PEFs) by National Authorities for Containment (NACs) in consultation with the GCC have now been established and published, and all countries intending to nominate and certify PEFs should establish NACs as a matter of urgency. Detailed guidelines for establishing and certifying PEFs have been produced, including milestones and key activities for NACs and PEFs.
- Poliovirus containment in the European Region is particularly onerous due to the number of vaccine production facilities located in the Region. Currently 13 Member States in the Region have indicated they intend to establish a total of 37 PEFs, 12 of which will be vaccine manufacturing facilities.
- A PEF ranking exercise is being undertaken to align risk mitigation priorities with current existing risk, align risk mitigation priorities with the containment “pre-requisites” to be met by the time of WPV eradication certification, and to help target country-specific advocacy efforts. The ranking protocol and rationale remains in development but a draft will soon be made available by the Containment Management Group (CMG) for discussion and comment.

- Other WHO Regions, including the Western Pacific Region, are facing many of the same or similar challenges to implementing containment as the European Region. Continued collaboration and the sharing of achievements, information and experience between the Regions is of obvious benefit to all.
- WHO has been responsible for a programme on laboratory biorisk management for several years, and that experience has been used in the determination of requirements for GAPIII. A series of guidance papers to help laboratories meet GAPIII requirements (Annex 6) while maintaining diagnostic capacity has been published by WHO, with three available to date. All countries are urged to make use of these guidance papers to accelerate progress towards achieving GAPIII requirements in polio diagnostic facilities (GPLN labs).
- Identification and appropriate handling of poliovirus potential infectious materials (PIM) has been a challenge for many years, and becomes more urgent with the requirement to contain materials potentially containing Sabin type 2 virus. WHO is preparing guidance on the risk assessment and risk stratification of PIMs together with guidance on risk mitigation strategies. This will be of importance to all diagnostic laboratories, particularly those that work with enteric and respiratory pathogens other than polio, as GAPIII requires that all PIMs either be destroyed or contained.
- Due to recent poliovirus outbreaks and emergency events, selected laboratories in the Region have now gained valuable experience in handling and holding PV2 infectious materials in compliance with containment requirements. The experience and lessons learned by these laboratories should be shared widely and used by all laboratories to develop preparedness plans for managing similar situations.
- Establishing a NAC and engaging in the process of proposing and certifying a PEF in compliance with GAPIII is a complex process that will hold significant challenges for many countries. Experience gained in the Region to date demonstrates that an active and systematic approach to the process is necessary, but that the process can be lengthy. Countries wishing to establish PEFs are urged to begin the process without delay.
- A recent spill of poliovirus infectious materials, and exposure of operators, at a vaccine production facility has provided valuable lessons in responding to containment breach events during the polio endgame period. The event, response mechanisms and lessons learned have been made public and all countries, particularly those hosting vaccine production facilities, should take note.
- An electronic platform is being developed for development of poliovirus inventories (e-inventory) to meet GAPIII containment requirements. This tool is designed primarily to facilitate implementation of Phase 1a and Phase 1b activities and will provide essential information on PEFs to the NPCC, together with documentation on the destruction and/or transfer of polio infectious materials. This tool is in the final stage of development.
- A revision of the polio LDMS is in development. This will be a web-based tool that will replicate the features of the current LDMS that will provide additional features, including bulk upload of data and a more comprehensive reporting function.

Recommendations

Laboratory

- WHO GPLN laboratories are urged to reach out to non-WHO diagnostic facilities in their countries using the professional networks available to them to ensure that all non-typed enterovirus isolates from patients with polio-compatible clinical conditions are screened to exclude poliovirus. All polioviruses detected must be forwarded to an accredited WHO polio laboratory for intratypic differentiation (ITD) and any further characterization required.
- All WHO laboratories should ensure complete and timely reporting of their findings via the WHO LDMS.
- To facilitate safe transport of infectious and potential infectious materials, laboratories should minimize transport of PV isolates for referral purposes and adopt the routine use of FTA cards. It is important to emphasize strict adherence to the WHO-recommended FTA sample application protocol to minimize a risk of residual infectivity.
- WHO laboratories are encouraged to pursue the implementation of ITD PCR in their laboratories in accordance with the regional implementation plan and to ensure that adequate human resources and funding is available for long-term routine ITD use.
- WHO laboratories are recommended to review and, if necessary, update their preparedness plans for sudden increase of sample numbers in the event of importation/outbreak detection.

Containment

- Facilities that have not been designated as Polio Essential Facilities (PEFs) but are currently in possession of PV2 materials should either ensure their documented destruction or transfer to a PEF as a matter of urgency.
- Non-PEF laboratories are encouraged to review their current practices and procedures to ensure compliance with the requirements described in Annex 6 of GAPIII. This review should also include current biosecurity provisions.
- Facilities that have already confirmed their intent to pursue GAPIII certification are encouraged to accelerate submission of their application for the Certificate of Participation to their respective National Authority on Containment (NAC).
- Designated PEFs are again urged to make themselves fully aware of the international requirements, including maintenance, in their hosting countries, of an effective national routine childhood polio immunization programme and high national population coverage with polio vaccine. PEFs and NACs must be fully aware of the exacting PEF containment certification requirements described in the Containment Certification Scheme to support the WHO Global Action Plan for Poliovirus Containment. Those facilities who would like to reconsider their earlier designation as PEF should communicate this decision to their respective National Poliovirus Containment Coordinator (NPCC).
- As recommended by the Regional Certification Commission, Member States that have not yet nominated a NPCC are urged to appoint this national focal point to ensure the effective communication and implementation of poliovirus containment activities.

- WHO guidance on the risk assessment and risk stratification of PIM will soon be available in draft format and laboratories are encouraged to provide feedback to WHO on the use of these guidelines.
- Laboratories are requested to provide comments and feedback on the proposed PEF risk ranking scheme and the PEF cost analysis exercise when the draft documents become available.

Annex 1. List of participants

Albania

Majlinda Kota
Virology Laboratory
Institute of Public Health

Austria

Birgit Prochazka
Federal Public Health Laboratory and Polio Reference Laboratory
Austrian Agency for Health and Food Safety

Belarus

Elena O. Samoilovich
Laboratory of Vaccine-Preventable Diseases
Republican Research and Practical Center for Epidemiology and Microbiology

Bulgaria

Lubomira Nikolaeva-Glomb
National Reference Laboratory of Enteroviruses
National Center of Infectious and Parasitic Diseases

Croatia

Irena Tabain
National Polio Laboratory
Croatian Institute of Public Health

Czech Republic

Petra Rainetová
National Reference Laboratory for Polio
National Institute of Public Health

Denmark

Thea Kølsen Fischer
Microbiological Diagnostics and Virology
Statens Serum Institut

John-Erik Stig Hansen
Centre for Biosecurity and Biopreparedness
Statens Serum Institut

Sofie Midgley
Microbiological Diagnostics and Virology
Statens Serum Institut

Finland

Soile Blomqvist
Viral Infections Unit
National Institute for Health and Welfare

France

Mael Bessaud
Biologie des virus entériques
Institut Pasteur

Bruno Lina
Laboratoire de Virologie du CHU de Lyon
Hopital de la Croix-Rousse

Georgia

Tamar Kutateladze
Polio National Laboratory
National Centre for Disease Control and Public Health

Germany

Sabine Diedrich
Regional Reference Laboratory for Poliomyelitis
Robert Koch Institute

Greece

Vasiliki Pogka
National Reference Laboratory for Measles and Rubella
Hellenic Pasteur Institute

Hungary

Ágnes Farkas
National Polio Laboratory
National Public Health Institute

Ireland

Jeff Connell
National Virus Reference Laboratory
University College Dublin

Israel

Itay Shimon Baror
Sheba Hospital

Merav Weil
Polio National Laboratory
Central Virology Laboratory

Italy

Paola Stefanelli
Department of Infectious Diseases
Instituto Superiore di Sanita

Kazakhstan

Gaukhar Nusupbayeva
National Reference Laboratory for Control of Viral Infections
Scientific and Practical center of sanitary and epidemiological expertise and monitoring

Latvia

Natalija Zamjatina
National Microbiology Reference Laboratory
Riga East Clinic Hospital

Lithuania

Algirdas Griskevicius
National Polio Laboratory
National Public Health Surveillance Laboratory

Netherlands

Erwin Duizer
Enteric Viruses
National Institute for Public Health and the Environment (RIVM)

Norway

Susanne Gjeruldsen Dudman
National Polio Laboratory
Norwegian Institute of Public Health

Poland

Magdalena Wieczorek
Enterovirus Laboratory of Virology Department
National Institute of Public Health- National Institute of Hygene

Portugal

Paula Palminha
Vaccine Preventable Diseases Reference Laboratory
National Institute of Health Dr Ricardo Jorge

Republic of Moldova

Ala Halacu
National Polio Laboratory
National Center of Public Health

Romania

Anda Baicus
Reference Laboratory of Microbiology
Cantacuzino National Institute of Research

Russian Federation

Maina Bichurina
Pasteur Institute in Saint-Petersburg

Liubov Kozlovskaya
Laboratory of Virology of Poliomyelitis and Enterovirus Infections
Chumakov Institute of Poliomyelitis and Viral Encephalitides of Russian Academy of Medical Sciences

Dmitrii Kudriatsev
Laboratory of Dangerous Virological Infections
Federal Center of Hygiene and Epidemiology

Ludmila Lebedeva
Laboratory of Virology
Center for Hygiene and Epidemiology in the Khabarovsk Territory

Galina Y. Lipskaya
A.N. Belozersky Institute of Physico-Chemical Biology

Nataliya Loginovskih
Virology Laboratory State Federal Institution of Health
Hygenic and Epidemiological Center in the Omsk Region

Iuliia Mikhailova
Northern Dimension Partnerships in Public Health and well-being
Federal Research Institute for Health Organization and Informatics

Zalimkhan Omariev
Biological Safety Division
Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing

Evgeniya Romanenko
Laboratory of Virology
Center for Hygiene and Epidemiology of Stavropol Kray

Tatiana Snitkovskaya
Laboratory of Virology
Hygenic and Epidemiological Center in the Sverdlovsk Region

Maria Yarmolskaya
Virology Unit of Microbiological Laboratory
Center for Hygiene and Epidemiology of Moscow

Serbia

Jelena Protic
National Polio Laboratory
Institute of Virology, Vaccines and Sera

Slovakia

Renata Kissova
National Reference Centre Poliomyelitis
Regional Authority of Public Health

Slovenia

Vesna Subelj
Laboratory for Public Health Virology
National Laboratory of Health, Environment and Food

Spain

Maria Cabrerizo
Instituto de Salud Carlos III

Sweden

Katherina Zakikhany
National Polio Laboratory
Public Health Agency

Switzerland

Rainer Gosert
Swiss National Polio Laboratory
University of Basel

Turkey

Gulay Korukluoglu
Virology Reference Laboratory
Public Health Institute

Zeynep Pinar Taskan
Sub-national Polio Laboratory
Izmir Regional Hygiene Institute

Ukraine

Iryna Demchyshyna
Virology Laboratory
Ministry of Health

Liudmila Kotlik
Virology Laboratory
Ministry of Health

United Kingdom

Robin Gopal
Scientific and Clinical Service
Public Health England

Javier Martin
Division of Virology
National Institute for Biological Standards and Control

Uzbekistan

Sultana Djemileva
Virology Laboratory
Republican Center of State Sanitary Epidemiological Surveillance

Partner Organizations

Anna Llewellyn
Global Poliovirus Laboratory Containment
US Centers for Disease Control and Prevention

Steve Oberste
Polio and Picornavirus Laboratory Branch
US Centers for Disease Control and Prevention

Katrin Leitmeyer
Preparedness and Response Unit
European Centre for Disease Prevention and Control

Jeff Partridge
Bill and Melinda Gates Foundation

Regional Certification Commission

Anton M van Loon
European Regional Certification Commission for Poliomyelitis Eradication

World Health Organization

Headquarters

Ousmane Diop
Surveillance, Labs and Data

Nicoletta Previsani
Research, Policy and Containment

Jacqueline Fournier-Caruana
Research, Policy and Containment

Regional Office for the Western Pacific

Varja Grabovac
Expanded Programme on Immunization

Regional Office for Europe

Florencia Biviano
Vaccine-Preventable Diseases and Immunization

Robb Butler
Vaccine-Preventable Diseases and Immunization

Sergei Deshevoi
Vaccine-Preventable Diseases and Immunization

Eugene Gavrilin
Vaccine-Preventable Diseases and Immunization

Shahin Huseynov
Vaccine-Preventable Diseases and Immunization

Maria Iakovenko
Vaccine-Preventable Diseases and Immunization

Theo Kaloumenos
Vaccine-Preventable Diseases and Immunization

Rapporteur

Ray Sanders

Interpreters

Olga Alexinskaya
Anna Nikolskaya

The WHO Regional Office for Europe

The World Health Organization (WHO) is a specialized agency of the United Nations created in 1948 with the primary responsibility for international health matters and public health. The WHO Regional Office for Europe is one of six regional offices throughout the world, each with its own programme geared to the particular health conditions of the countries it serves.

Member States

Albania
Andorra
Armenia
Austria
Azerbaijan
Belarus
Belgium
Bosnia and Herzegovina
Bulgaria
Croatia
Cyprus
Czechia
Denmark
Estonia
Finland
France
Georgia
Germany
Greece
Hungary
Iceland
Ireland
Israel
Italy
Kazakhstan
Kyrgyzstan
Latvia
Lithuania
Luxembourg
Malta
Monaco
Montenegro
Netherlands
Norway
Poland
Portugal
Republic of Moldova
Romania
Russian Federation
San Marino
Serbia
Slovakia
Slovenia
Spain
Sweden
Switzerland
Tajikistan
The former Yugoslav Republic of Macedonia
Turkey
Turkmenistan
Ukraine
United Kingdom
Uzbekistan

World Health Organization
Regional Office for Europe

UN City, Marmorvej 51, DK-2100 Copenhagen Ø, Denmark
Tel: +45 45 33 70 00 Fax: +45 45 33 70 01
Email: eucontact@who.int
Website: www.euro.who.int