Fifteenth Informal Consultation of the Global Polio Laboratory Network
Summary of Discussions and Recommendations

An informal consultation of the Global Polio Laboratory Network (GPLN) was held from 23 to 25 June 2009 at WHO's Headquarters in Geneva, Switzerland. Participants represented 20% of the 144 network laboratories and all 6 WHO regions. The consultation reviewed global trends in the detection of wild and vaccine derived polioviruses, workload, the GPLN's quality assurance programme and progress towards the development of new laboratory diagnostic procedures.

In 2008 the network analysed approximately 165,000 faecal samples from AFP cases and 14,500 samples from non-AFP sources, which represented a 6% overall increase in workload compared to 2007.[1] Results from the GPLN identify the geographical locations where wild polioviruses are found, and, comparative analysis of the nucleotide sequence of the VP1 region of the viral genome allow inferences to be made about genetic relatedness and the possible transmission links among different isolates.

Detection of wild polioviruses

Transmission of four genotypes has continued since 2005: two genotypes (designated West Africa B types 1 and 3 i.e.WEAF-B PV1 and WEAF-B PV3) are endemic to Nigeria, and two other genotypes (South Asia types 1 and 3 i.e. SOAS PV1 and SOAS PV3) are endemic to Afghanistan, India and Pakistan. The aforementioned 4 countries have never interrupted transmission of their endemic genotypes. In 15 other countries wild polioviruses found in 2008 belonged to genotypes found in Nigeria (11 countries) or India (3 countries) or both (1 country). WEAF-B PV1 and WEAF-B PV3 genotype viruses were both found in Benin, Chad, Niger and Sudan in 2008, whereas seven other countries (Burkina Faso, Côte d'Ivoire, Central African Republic, Ethiopia, Ghana, Mali and Togo) had only WEAF-B PV1. Viruses belonging to the SOAS PV1 and SOAS PV3 genotypes were found in Angola and Democratic Republic of Congo (DRC) in 2008 and 2009, and only SOAS PV3 genotype was found in Nepal in the same year. Unusually, there were two "silent" importations of WEAF-B PV1 and SOAS PV1 viruses into Egypt in 2008: two separate single sewage samples yielded these viruses, and no poliomyelitis cases were found during follow up investigations.

Between January and June 2009, the GPLN has detected WPV in 19 countries: 12 have represented continuation of previously detected transmission. New detections of WEAF-B PV1 have been made in 4 countries (Guinea, Liberia, Kenya, Uganda) and SOAS PV3 virus imported into Angola in 2008 that spread into Democratic Republic of Congo in the same year. WEAF-B PV3 viruses spread from Chad to Central African Republic in 2009.

[1] Workload increases were 20% in the region of Africa (AFR), 52% in Europe (EUR), 5% in South East Asia (SEAR) and 2 % in the Western Pacific (WPR). Workload remained unchanged in the region of the Americas (AMR) and declined by 9% in the Eastern Mediterranean region.

Detection of Vaccine Derived Polioviruses, VDPV

The GPLN reported vaccine derived polioviruses (having > 1% VP1 nucleotide sequence divergence from the Sabin parental strain) from several locations in 2008 and 2009. A VDPV serotype 2 (VDPV2) outbreak, on-going since 2006, continued in Nigeria. Failure to interrupt this outbreak jeopardizes the most significant achievement of the Polio Eradication Initiative to date, the global interruption of transmission of wild serotype 2 viruses since 1999. In terms of virology, the VDPVs in Nigeria show reversion of mutations associated with attenuation of the Sabin virus, and transmission now represents uninterrupted circulation and evolution of the wild serotype 2 parental strain. A total of 269 cases have been reported since the start of the VDPV2 outbreak in Nigeria, and 156 of these cases were reported since January 2008. Separate VDPV2 outbreaks were detected in 2008 and continued into 2009 in the Democratic Republic of Congo (DRC, 16 cases?) and Ethiopia.

VDPVs were also isolated from immunodeficient persons: VDPV1 from a single immunodeficient person in Argentina in 2009, VDPVs retrospectively reported for a patient in the United States (VDPV 2 with OPV exposure 13 years before) and another in the United Kingdom (VDPV3 with onset in 2000). VDPVs of different serotypes, and not associated with outbreaks or immunodeficiency (and therefore considered to be of ambiguous origin, designated aVDPV), were isolated from AFP cases in several other locations. aVDPV2 was found in 2008 in Angola (2), Russian Federation (1) and Somalia (1), and in 2009 from Guinea (1) and India (1). aVDPV3 was found in a single AFP case in Malawi in 2008. aVDPVs were also identified in sewage specimens in 4 countries, VDPV2 in two specimens in Egypt, in Estonia (2 specimens) and in Israel (multiple specimens); no paralysed persons were found during follow up investigations. In Estonia sewage specimen from September and December 2008 yielded VDPV2 and VDPV3, respectively, while in Finland a single sewage specimen during December 2008 yielded both VDPV1 and VDPV2.

Laboratory Quality Assurance

WHO continues to administer a laboratory accreditation programme for the GPLN and monitors performance for virus isolation in cell culture and intratypic differentiation (ITD) of polioviruses as Sabin-like or wild. Accreditation is based on annual performance reviews, most frequently conducted on-site in laboratories by expert virologists. Laboratories are required to meet pre-set standards for accuracy and timeliness of results. Ninety five percent of the 144 network laboratories were fully accredited in 2008. Six provisionally accredited laboratories generally met required standards for accurate results, but had some other deficiency of performance. Two non-accredited laboratories failed proficiency tests and are testing samples in parallel with accredited reference laboratories, while resolving performance concerns and one sub-national laboratory in South America has been inactive for a few years.

It was noted with satisfaction that a new algorithm for more rapid PV detection has now been implemented in 4 WHO regions, Africa (AFR), the Americas (AMR), the Eastern

Mediterranean (EMR), and South East Asia (SEAR), and that these regions evaluated a newly developed proficiency test for this algorithm for the first time in 2008. Laboratories in the 4 regions fully met the timeline of 14 days for virus isolation. ITD reporting within 7 days of receipt of isolates was achieved in AFR, AMR and EMR but not in SEAR in 2008. High workload laboratories in SEAR face serious challenges in meeting the ITD reporting targets, and opportunities for re-distribution of workload to improve efficiency will depend on completion of on-going work to increase ITD capacity in the region. Performance must be strengthened in some laboratories in AMR to address weaknesses identified through proficiency tests done in 2008. Implementation of the algorithm has started in the Western Pacific (WPR) in 2009.

The consultation reviewed plans to develop and implement a quality assurance programme for laboratories that perform nucleotide sequencing of PVs. This is proposed to involve standardization of training of personnel and laboratory processes, proficiency testing, and extending the WHO accreditation programme to include evaluation of sequencing performance.

New real time PCR (rRT-PCR) procedures for ITD and screening for VDPVs

Two separate real-time PCR (rRT-PCR) assays have been developed by scientists at the United States Centers for Diseases Control and Prevention (CDC) for ITD and for screening for VDPVs. These assays were validated at CDC and are under evaluation in field conditions in 6 global specialized laboratories, 2 high-workload regional reference laboratories (RRLs) located in South Africa and Pakistan that serve polio endemic regions, and in the RRL in Australia that serves WPR, a non-endemic region . The field evaluation involves retrospective testing of known VDPVs, screening of previously reported Sabin-like PVs for VDPV, and prospective testing of newly identified PVs using an rRT-PCR test algorithm for both ITD and VDPV screening. The consultation received the following feedback from those involved in the field evaluation:

1. **Operational issues.** A variety of real-time PCR instruments have been used. Laboratories that used ABI 7500 or Stratagene MX3000/3005 easily implemented the procedure while those using the Roche LightCycler or Corbett Rotor-Gene required changes to the cycling programmes (mainly to temperature ramp times) for successful amplification by some primer sets. Amplification could not be obtained for degenerate primers when error-correcting reverse transcriptase was used. The assays work well with both virus culture supernatants and extracted RNA. Routine use of RNA extraction, however, has been estimated to double the cost per isolate analysed. One laboratory provided feedback that chloroform treatment of isolates followed by repeat rRT-PCR resolved some invalid results. Parallel testing of isolates using current (conventional RT-PCR and ELISA) and rRT-PCR ITD algorithms demonstrated a comparability of results of > 95% in several laboratories. The evaluation is continuing in several laboratories.
2. **Retrospective screening of known VDPVs using rRT-PCR assays.** The VDPV rRT-PCR assay targets key sites in the VP1 and 3D regions of the Sabin PV genome, with NSL (negative) results expected for VDPVs with VP1 primers. Data from the evaluations showed that a NSL (negative) result using 3D primers

for PVs, indicating recombination with viruses other than the parental Sabin strain, does not provide additional sensitivity for detection of VDPVs. Isolates from the following past cVDPV outbreaks were screened by rRT-PCR methods: Cambodia (VDPV3), Madagascar (VDPV2 and VDPV3 outbreaks of 2005); VDPV1 outbreaks in China, Hispaniola, Indonesia, Philippines (VDPV1); and VDPV2 outbreaks in Egypt and Nigeria. iVDPVs from "Taiwan, China" and past aVDPVs from Egypt, Japan, Czech Republic, Slovakia and Estonia were also tested. Feedback to CDC from the field evaluation allowed for minor modifications to be made to improve reagent performance.

3. **Retrospective screening for VDPVs among previously reported SL viruses.** Network laboratories in India, Pakistan, South Africa and the United States have done this work in the field evaluation. In India, priority was given to serotype 2 and 3 viruses detected in Uttar Pradesh and Bihar in 2007 and 2008, and no VDPVs were found from this period. The RRL in Pakistan tested SL viruses of all 3 serotypes that had been isolated from AFP cases in 2007 and 2008, and no VDPVs were found retrospectively. CDC-United States tested SL viruses from Nigeria for the period 2006 to 2009, and the previously mentioned cVDPV outbreak isolates were found. In South Africa, the workload for retrospective testing is large, as only 1 laboratory (situated at the National Institute of Communicable Diseases, NICD, Johannesburg) serves the region. Over 1,900 SL viruses from countries other than Nigeria have been screened by NICD; all but 50 of the viruses were reported since 2005. aVDPVs were retrospectively detected in Democratic Republic of Congo (DRC; 2 in 2005 and 2 in 2007), and in Somalia (1 in 2005). The cVDPV outbreak viruses in DRC and Ethiopia in 2008/9 were also detected by NICD during the evaluation of the rRT-PCR. The consultation learned that thirty percent of the cVDPV viruses detected in Nigeria and all of the retrospective aVDPV detected to date in Africa had been missed using the GPLN's test algorithm that used conventional RT-PCR and ELISA for ITD, followed by VP1 nucleotide sequencing of isolates that did not react as SL in the 2 ITD tests.

The GPLN has begun preparatory work for full implementation of rRT-PCR methods. Approximately 25 laboratories already have access to equipment and funding has been secured from Rotary International to provide rRT-PCR equipment to another 25 laboratories and for training of personnel. Two training workshops were conducted in the regions of EMR and SEAR in 2008, and similar workshops are scheduled in the 4 remaining regions by the end of 2009, with a view to having 50 laboratories fully implementing rRT-PCR methods by end 2010. Some changes will be required in proficiency testing and data management in all regions when rRT-PCR tests are introduced.

Other products under development for use in the GPLN

The network is exploring alternative methods to reduce the biorisk and cost associated with inter-laboratory shipping of PV isolates for ITD and sequencing. Participants were informed of the progress in evaluating procedures for inactivation of isolates spotted onto

special filters. Prevention of cross-contamination between samples remains a challenge with this method, and alternative methods of PV inactivation are being evaluated.

One alternative polio diagnostic approach under development for use in the GPLN involves direct detection of PVs in faecal samples using a test algorithm based only on molecular methods. While nucleic acid based technologies exist for PV detection, the challenge to the GPLN has been to identify highly sensitive tests to detect wild PVs that vary in genotype and demonstrate highly genetic variability and the need for test protocols that can be easily implemented in a variety of resource settings. A molecular-based test algorithm can potentially revolutionize testing in the GPLN and accomplish a substantial reduction in analytical time through elimination of virus isolation in cell culture, thereby also reducing the risks for breaches of containment when live viruses are replicated. A conceptual framework has been prepared for validating this proposed approach.

The consultation reviewed test development and validation results for IgM and IgA ELISA assays that are under development for use in non-OPV using settings. Functional assays were described that can detect IgM and IgA in individuals that have been immunized with either OPV or IPV. The remaining technical challenges are evaluation of IPV as assay antigen, to eliminate the containment issues associated with use of live virus antigen in non-OPV using settings, and the optimization of assay positive controls. Evaluation and validation studies are ongoing.

Investigations are also on-going to identify ways to modify current laboratory approaches used for analysing sewage samples for PV detection. The most frequently used procedures are labour- and workload-intensive, especially in OPV using countries where programmatically important viruses (wild PV and VDPV) must be isolated from samples having an excess of OPV strains, and PVs in mixtures must be separated before characterization. The availability of more efficient methods will allow for expansion of the number of countries that test sewage waters for PV. There have been several examples of programmatically important information being obtained from testing of sewage samples for PVs. At the current stage of the polio eradication programme, this supplementary surveillance approach may be useful where AFP surveillance standards are known or suspected to be sub-standard, especially where there is interest in investigating the possibility of missed transmission in polio-free regions within endemic countries. An immediate priority is investigation of whether incubation at elevated temperature can be used to select polioviruses of programmatic interest, because this can potentially reduce workload in laboratories that process environmental samples.

CDC-Atlanta is developing automated informatics workflows to facilitate the processing, analysis and presentation of PV sequence data for Programme needs. The goal is to create distributable programs that can be used in GPLN sequencing laboratories.

Other contributions of the GPLN

Several network laboratories are contributing to research, development and evaluation of new polio vaccines or changes in immunization policy. Mention was made of such contributions by laboratories in Bandung-Indonesia, Cuba, Mumbai-India, the Netherlands, United Kingdom and United States.

Network laboratories are also contributing to detection of influenza viruses. Individual countries decide on which facility supports influenza diagnoses. However, WHO/AFR has adopted a policy to encourage integration of laboratory diangnosis for influenza viruses into existing polio laboratories. In other regions some GPLN facilities are sharing personnel or equipment for detection of influenza or other vaccine-preventable diseases (e.g. measles, yellow fever, Japanese Encephalitis), but these contributions are not always well documented.

Programme colleagues should be encouraged to attend the annual GPLN Consultation, to facilitate communication of critical issues.

Recommendations

1. The Proficiency Test (PT) developed for the new test algorithm for rapid poliovirus detection should be continued in the GPLN, retaining a similar level of complexity, scoring system, passing score and timeline for reporting. The proficiency panel was successful in identifying laboratories with performance concerns. Regional laboratory coordinators should continue close follow-up to assist laboratories that fail the proficiency test preferably accompanied by on-site review, and, for all laboratories with a suboptimal score, to identify and correct problem work practices. Laboratories using the new algorithm should be evaluated using the appropriate PT and reporting timeline of 14 days for virus isolation and 7 days for intratypic differentiation.
2. The rRT-PCR algorithm should be fully implemented in current network ITD laboratories that are not using sequencing for ITD to replace the "conventional PCR plus ELISA" algorithm for ITD by the end of 2010.
3. Appropriate arrangements should be made for adjustments in proficiency testing and data management and for phasing out of the current algorithm of conventional ITD by December 2010.
4. For rRT-PCR assays, the sample of choice should be the cell culture supernatant of the isolate. RNA extraction and repeat testing should be done for only those isolates that initially give invalid rRT-PCR test results.
5. During the transition phase, and until accredited to perform rRT-PCR tests, laboratories will be requested to perform parallel testing of PVs for ITD and VDPV screening using conventional and rRT-PCR algorithms. Guidance will be provided on the number of isolates to be tested. Any isolates giving discordant results in either of the 2 algorithms should be referred for sequencing. Results should be analysed to give estimates of percentage comparability of results between the traditional and rRT-PCR algorithms.
6. GPLN facilities in Finland and the United States should continue the development of protocols for non-infectious shipment of isolates. These protocols should be

pilot tested by end of 2009 with a view to wider scale introduction in the GPLN in 2010.

7. Laboratories should continue the developmental work on a test algorithm based on only molecular or other non-cell cultures based procedures with an aim to complete the development and validation of approaches and report on progress by June 2010.
8. The consultation endorsed the approaches being taken by WHO/HQ and CDC-Atlanta to develop a quality assurance programme for sequencing laboratories, recommending that the draft accreditation checklist presented at the meeting, incorporating suggestions from the group by end of July 2009, be pilot tested in 3 laboratories and that a proficiency test programme be established by June 2010.
9. Protocols for sequencing should be standardized to simplify technology transfer and ensure comparability between laboratories. Training modules based on the standardized protocols should be developed. Systems for quality assurance and proficiency testing for sequencing laboratories should be finalized and evaluated by March 2010. Standard definitions for genotypes and genetic clusters should be applied. Development should continue on automated methods to process, analyze, and present sequence data.
10. CDC and RIVM should continue development of IgM and IgA assays These assays should be evaluated under field conditions by end 2010 and the possibility of linking these evaluations to planned studies to evaluate new polio vaccines should be explored.
11. Recognizing that environmental surveillance is increasingly more important in the late phase of eradication, studies should continue with the aim to provide data on simplified and more efficient processing of sewage concentrates. Data should be analysed to determine whether workload for PV characterization can be reduced without compromising sensitivity for detection of programmatically important PVs.
12. Future Consultations should include laboratory management among the agenda topics.