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1. INTRODUCTION

The fourth intercountry meeting of directors of poliovirus laboratories in the WHO Eastern Mediterranean Region was held in Muscat, Oman, from 16 to 18 May 2000. The meeting was attended by laboratory directors from Egypt, Islamic Republic of Iran, Jordan, Kuwait, Morocco, Oman, Pakistan, Saudi Arabia, Sudan, Syrian Arab Republic and Tunisia. Participants also included scientists from the Centers for Disease Control and Prevention (CDC), United States of America; National Institute of Public Health and the Environment (RIVM), Netherlands; National Public Health Institute, Finland; National Institute for Biological Standards and Control (NIBSC), United Kingdom; and WHO headquarters and the Regional Office for the Eastern Mediterranean.

Dr Ahmed Abdel Kader Al Ghassani, Undersecretary in the Ministry of Health, Oman, welcomed the participants to the meeting. He stated that the last poliomyelitis case in Oman has been in 1993 in a 10-month old child. Strong support for the poliomyelitis eradication efforts had come from His Excellency Sultan Qaboos and through both governmental and nongovernmental agencies. The immunization programme had started in 1980, and 3 doses of OPV were given to children in infancy through the routine immunization programme. National immunization days (NIDs) were conducted in 1992 and 1994 after outbreaks of poliomyelitis, and had been conducted annually in Oman since 1995 as a supplementary immunization strategy with a sustained coverage of over 95%. Strong laboratory support was available at the Central Public Health Laboratory of Oman, and the laboratory had been fully accredited by WHO. Thanks were extended to the representatives of international agencies for their continued support to laboratories of the Region. Such international collaboration was exemplary and a model for laboratory support for other communicable diseases. Dr Al Ghassani stated that the achievement of the goal of poliomyelitis eradication was imminent and that it was appropriate that the agenda for the meeting included discussions on environmental surveillance and containment of laboratory stocks of infectious materials, since both of these issues would be relevant in the final stages of eradication. Dr Al Ghassani thanked the participants for their commitment to the programme of poliomyelitis eradication and stated that he looked forward to the time when the Region could be certified as polio-free.

Dr Ibrahim Abdel Rahim, WHO Representative, Oman, delivered a message to the participants on behalf of Dr Hussein A. Gezairy, WHO Regional Director for the Eastern Mediterranean. He thanked the Ministry of Health for hosting the meeting, welcomed the participants and thanked representatives of the various international agencies for their support of the network of 12 laboratories involved in the regional poliomyelitis eradication programme. Dr Abdel Rahim stated that considerable progress was being made in the WHO Eastern Mediterranean Region towards achieving the eradication goal. Up to December 1999, no poliomyelitis cases had been reported for 3 or more years in 14 of the 23 Member countries. Extraordinary efforts were being made in the remaining countries to interrupt indigenous wild virus transmission through improvements in surveillance for cases of acute flaccid paralysis (AFP) and immunization. The virology laboratory network continued to provide support to the surveillance activities and had detected wild polioviruses in 7 countries in 1999. During the final stages of the eradication programme, laboratories would be required to sustain excellence in performance and to assist the Region in ensuring that virus isolates and other infectious materials were properly handled and stored to prevent them from serving as a source of infection for individuals or communities. Dr Abdel Rahim wished the

participants success in their deliberations and in the formulation of recommendations to further strengthen laboratory performance.

Dr Suleiman A. L. Busaidy, Director of the National Poliovirus Laboratory, Oman was elected as Chairman of the meeting. The programme and list of participants are included in Annexes 1 and 2, respectively. Recommendations from the third intercountry meeting of directors of poliovirus laboratories are included in Annex 3.

2. OVERVIEW

2.1 Implementation of the recommendations of the third intercountry meeting of directors of poliovirus laboratories in the Eastern Mediterranean Region

Dr Esther de Gourville, Virologist, WHO/EMRO

Recommendations of the previous meeting were targeted at improving performance related to sample referral; use and interpretation of molecular epidemiology data; data management; and coordination between virologists and EPI teams (see Annex 3). The recommendations on molecular epidemiology have been fully implemented, as have those related to data management. Laboratory and surveillance coordination has generally improved in several countries. However, in three countries coordination still requires strengthening as virologists are not involved in, or informed about, the final classification of individual AFP cases, nor are they involved in planning for immunization campaigns.

The majority of the previous recommendations related to sample referral have been implemented. However several laboratories failed to refer poliovirus isolates to reference laboratories within the recommended 14 days of detection for characterization as wild or vaccine-like. Individual laboratories varied in the reasons for failing to achieve the 14-day target, and the Regional Office is assisting laboratories on an individual basis to resolve problems. Delays in confirmation of wild viruses can seriously jeopardize the poliomyelitis eradication programme as they may facilitate undetected virus transmission and result in failure to implement effective public health responses targeted at communities at highest risk of poliovirus infection.

2.2 Global status of poliomyelitis eradication

Dr Raymond Sanders, Scientist, WHO/HQ

There have been significant achievements towards attaining the goal of global poliomyelitis eradication. Approximately 7000 poliomyelitis cases were reported in the world in 1999, compared to 350 000 cases in 1988 when the eradication progamme began. Indigenous wild poliovirus transmission was interrupted in the WHO Region of the Americas in 1991 and the Western Pacific Region in 1997. As of December 1999, more than 1 year had passed without any wild viruses being detected in the European Region. The transmission of poliovirus serotype 2 appears to be on the decline and the most recent detection of this serotype was in September 1999 in Uttar Pradesh, India. Transmission of poliovirus serotypes 1 and 3 continues in a few countries. However, several of the remaining large poliomyelitis endemic countries have intensified efforts to achieve the eradication goal. For example, in the winter of 1999, India immunized close to 150 million children with 4 doses of oral poliovaccine (OPV) which was a significant achievement since India accounted for 40% of all

reported poliomyelitis cases in 1999. Similarly, in Pakistan and Nigeria, millions of children were reached through house-to-house immunization across large geographic areas. The United Nations negotiated for and obtained days of tranquillity in the Democratic Republic of the Congo in 1999, and the cessation of hostilities allowed health officials access to immunize approximately 8 million children.

There have also been significant laboratory achievements. With the exception of the Democratic Republic of Korea, all countries of the world have access to laboratories for virological investigation of AFP cases. Over 85% of the laboratories are accredited by WHO and over 50 000 faecal samples were tested in 1999. All wild viruses detected in 2000 will be genetically characterized.

Despite significant progress, it is unlikely that global poliomyelitis eradication will be achieved by the end of the year 2000. Twenty-four countries reported wild viruses in 1999 and 6 others are thought to have ongoing virus circulation because many clinical cases continue to occur in settings of poor surveillance and inadequate collection of samples for virology investigations. Additionally, importations of wild viruses into 3 previously polio-free countries were documented in 1999. This emphasizes that all countries of the world will remain at risk for poliomyelitis until the disease is eradicated globally. A strategic plan has been developed for the period 2000 to 2005 to meet the remaining challenges. Acceleration of activities in remaining poliomyelitis endemic areas will focus on: increasing immunization coverage through more frequent, high quality, supplementary immunization activities; improving AFP surveillance, particularly in the WHO African Region, where 20 of 46 countries are endemic or probably endemic, and in the WHO South East Asia Region where wild polioviruses were detected in 1999 in India, Bangladesh and Nepal; strengthening of routine immunization programmes; containment of wild polioviruses within laboratories; and implementing research to achieve consensus on stopping immunization.

2.3 Regional status of poliomyelitis eradication

Dr Faten Kamel, Medical Officer, WHO/EMRO

Regional achievements in poliomyelitis eradication have been reflected in all of the critical eradication strategies and in almost all of the countries of the Region. Routine OPV3 coverage of above 90% has been reached in 16 countries of the Region. Improvement in routine OPV coverage to at least 80% is still needed in Afghanistan, Djibouti, Pakistan, Somalia, Sudan and the Republic of Yemen.

During 1999 supplementary immunization activities were implemented in all Member States except Cyprus, and high coverage was achieved. The Islamic Republic of Iran and Tunisia conducted only sub-national immunization activities in border and high-risk areas. Most significantly, two national immunization days (NIDS), delivering 4 doses of OPV per child, rather than the customary one NID (delivering 2 doses of OPV per child) were conducted in 3 countries in response to different factors that threaten progress towards achieving the eradication goal: in Egypt when a few wild virus cases occurred after some months without cases; in Afghanistan to compensate for a very low routine OPV coverage; and in Iraq in response to an outbreak of poliomyelitis that started in May 1999. Pakistan, Sudan and the Syrian Arab Republic conducted mopping-up operations in high-risk and/or border areas, in addition to conducting one NID. During the house-to-house operations in

Pakistan, almost 11 million children under 5 years of age were immunized. There was improvement in access to some population groups in Afghanistan and Somalia in 1999. The second round of NIDs in Afghanistan reached almost all areas in the country. During the first round of NIDs in Somalia some areas were not included due to security concerns, but almost all of these areas were covered during the second round (with the exception of Mogadishu), and an additional round was later implemented in these areas. Coordinated NIDs and mopping-up campaigns continued in an effort to prevent cross-border wild poliovirus transmission between Afghanistan, Islamic Republic of Iran and Pakistan, and between the Islamic Republic of Iran, Iraq, Syrian Arab Republic and Turkey. In addition to these Operation MECACAR-supported activities, different regional and interregional epidemiological blocks also coordinated immunization and surveillance activities, e.g. countries of the Gulf Cooperation Council, Maghrebian Union and South Asian Association for Regional Cooperation. Attention is currently being focused on collaboration with the WHO Regional Office for Africa to achieve coordination among the countries of the Horn of Africa, especially Djibouti, Eritrea, Ethiopia, Somalia and Sudan.

During 1999, there were improvements in AFP surveillance throughout the Region. For the first time the regional AFP detection rate exceeded 1 per 100 000 children under 15 years (1.15) as compared to rates of 0.71 in 1996, 0.85 in 1997 and 0.88 in 1998. AFP rates of more than 1 per 100 000 children under 15 years were reported from 15 countries and rates between 0.5 and 1 per 100 000 were reported from 6 countries. It is of concern that AFP surveillance is not yet covering all areas in Afghanistan and Somalia, mainly because of restricted access to populations in areas of conflict. Another concern is that, in 1999, Djibouti and Sudan had AFP detection rates of less than 0.5 per 100 000. A challenge in several countries is that the quality of AFP surveillance is compromised by inability to collect adequate stools from at least 80% of all investigated AFP cases. Only 7 of 23 countries achieved this level, and the regional average in 1999 was 69% for collection of adequate stools, too low to provide reassurance about the absence of wild polioviruses in some areas. As part of acceleration of efforts, active AFP surveillance has been strengthened in the Region. National surveillance officers have been recruited in some countries and provision of technical support from the WHO Regional Office has been strengthened. Although most Member States are making significant progress in AFP surveillance, a lot remains to be done to achieve the quality of surveillance required to target efforts and to certify eradication.

There were 862 poliomyelitis cases reported in the Region in 1999. This number was greater than the 554 cases reported in 1998, mainly because of the occurrence of poliomyelitis outbreaks in Afghanistan and Iraq and the improvement in AFP surveillance in several countries. A little less than 60% of all reported poliomyelitis cases in the Region were from Pakistan (507), and the remaining cases were reported from Afghanistan (150), Iraq (88), Sudan (60), Republic of Yemen (24), Somalia (19), Egypt (9), Islamic Republic of Iran (3), Syrian Arab Republic (1) and Djibouti (1). Of the 23 countries of the Region, 13 reported zero cases and 10 of them have reported zero cases for 3 or more consecutive years.

The regional activities for certification of poliomyelitis eradication are gaining momentum. In November 1999 the Regional Commission for Certification of poliomyelitis eradication reviewed the reports from 6 countries, and all but one report were found

satisfactory. The committee advised the representatives of the country with the unsatisfactory report to revise and re-submit the report in the year 2000.

Despite significant achievements, there are some constraints that must be overcome to achieve poliomyelitis eradication in the Region. These include problems in countries with civil unrest that obstructs routine immunization and surveillance activities. Extraordinary efforts will be required from United Nations and other agencies to negotiate for peace and to increase human and financial resources and multi-agency coordination for effective implementation of immunization and surveillance activities. Some other countries have low routine immunization coverage, gaps in supplemental immunization quality and/or inadequate AFP surveillance, particularly in sub-populations that are difficult to reach. Innovative strategies will be required to overcome these difficulties. Efforts continue to ensure that the commitment of governments and international agencies is translated into effective action and that deficiencies in management or implementation are addressed in each country, even down to the local administrative level. These are critical issues because the poliomyelitis eradication programme has entered its final and most difficult phase.

2.4 Virological surveillance in the Eastern Mediterranean Region

Dr Esther de Gourville, Virologist, WHO/EMRO

Virology results are increasingly being used for final classification of AFP cases in the WHO Eastern Mediterranean Region. Twenty-three countries referred samples to WHO network laboratories in 1999 compared to 20 countries in 1998 and 16 in 1997. Fifteen countries are using virology results for final classification of AFP cases. Established standards for laboratory performance, as well as technical and managerial procedures are evaluated through an accreditation programme that requires an annual review of performance with on-site observation of work practices as appropriate. Of the 12 regional laboratories, 9 were fully accredited in 1999, and 3 were only provisionally accredited.

In 1999, the Region reported a total of 3039 AFP cases and virology investigations were done on 91% of these cases. Overall, 6467 samples were tested, comprising 5507 stools from AFP cases and 960 samples collected from contacts, the environment or other sources. Adequate stool samples (i.e. 2 samples collected at least 24 hours apart and within 14 days of paralysis onset) were collected from only 69% of reported AFP cases; this fell short of the performance target of 80%. Only 50% of samples were received in laboratories within 3 days of collection from patients, and failure to meet the performance target of 80% generally resulted from unavoidable delays as samples are shipped via commercial couriers to distant laboratories. Despite transport delays, 93% of all samples were received in laboratories in good condition. Virology results were reported within 28 days of sample receipt for 80% of AFP cases, and, non-polio enteroviruses (NPEV) were isolated from 8.4% of all samples tested. It was very encouraging to see that improvement was made in several aspects of surveillance and laboratory performance. However it was of concern that some deficiencies still existed in sample collection and referral to laboratories, as these weaknesses have the potential to decrease the reliability of negative virology results. Failure to isolate viruses may be because samples are collected from patients at a time of low virus excretion, or because viruses are inactivated when samples are stored under poor conditions in the field.

Wild polioviruses were isolated from 54% of the 862 confirmed poliomyelitis cases in the Region in 1999, with the remaining cases confirmed on the basis of clinical findings. Wild viruses were detected in 7 countries. Serotype 1 occurred in Afghanistan (46 cases), Egypt (7 cases), Islamic Republic of Iran (3 cases), Iraq (68 cases), Pakistan (273 cases), Sudan (7 cases), and Syrian Arab Republic (1 case). In Islamic Republic of Iran, 2 cases were imported since disease onset occurred in Afghanistan, and the third case had links to Afghanistan based on epidemiological data as well as the genetic characteristics of the virus isolate. Genetic studies on the serotype 1 viruses of 1999 showed that, except for Syrian Arab Republic, all other viruses were indigenous to the Eastern Mediterranean Region. The Syrian Arab Republic polio 1 virus was over 98% related to viruses detected in Bihar, India, in 1999, and investigations continue to determine the possible exposure history of the Syrian Arab Republic case. Polio 1 viruses from Iraq in 1999 were genetically derived from a single virus genotype that was transmitted previously in both Iraq and Turkey. Prior to 1999, viruses from this genotype had last been detected in Iraq in 1997, and Turkey in November 1998.

Wild poliovirus serotype 2 was not isolated in the Region in 1999 and was last detected in Pakistan and Afghanistan in 1997. In 1999 wild poliovirus serotype 3 was isolated from cases in Afghanistan (16 cases), Egypt (2 cases), Pakistan (67 cases), Somalia (1 case) and Sudan (2 cases). The detection of type 3 viruses in Somalia and Sudan was probably a direct result of the improvements in AFP surveillance and sample handling, as prior to 1999 this serotype had not been detected for more than 3 years in either country. The isolation of type 3 viruses in Egypt in 1999 was of concern as type 3 had last been detected in Egypt in December 1996. Genetic sequencing ruled out the possibility importation of the type 3 virus into Egypt, as the viruses were shown to be indigenous to Egypt. Further, the Egyptian type 3 viruses were distinct from those found in Afghanistan, Pakistan and Sudan. The type 3 viruses in Egypt occurred in the same limited geographic foci in which wild polio 1 cases were also detected in 1999, and these areas are being targeted in house-to-house immunization campaigns to interrupt virus transmission.

Network laboratories continued to detect wild polioviruses in 2000 and, up to March 2000, wild polioviruses were detected in Afghanistan, Egypt, Iraq, Pakistan, Somalia and Sudan.

3. LABORATORY REPORTS ON COUNTRIES WITH WILD POLIOVIRUSES

3.1 Afghanistan and Pakistan

Dr Humayun Asghar, National Institutes of Health, Pakistan

During 1999 the regional reference laboratory (RRL) in Pakistan tested 2791 samples comprising 2372 samples from 1255 AFP cases from Pakistan and 419 samples from 213 cases from Afghanistan. The proportion of AFP cases with samples collected within 14 days of paralysis onset was 80% for Pakistan and 63% for Afghanistan. Over 80% of all samples were received in the laboratory in good condition, although only 42% and 27% of samples from Pakistan and Afghanistan, respectively, reached the laboratory within 3 days of collection from the patients. Results were reported within 28 days for 79% of samples from Pakistan and 70% of samples from Afghanistan. Non-polio enteroviruses (NPEV) were isolated from 6% of Pakistan samples and 7% of Afghanistan samples.

In 1999 the laboratory isolated polioviruses from 960 samples, 817 of which were from Pakistan and 143 from Afghanistan. Only 23% (216) of poliovirus isolates were Sabin vaccine-like, all others (744) were wild viruses. All poliovirus serotype 2 isolates were Sabin-like. The distribution of wild virus isolates was as follows: 505 of type 1 and 122 of type 3 from Pakistan; and 86 of type 1 and 31 of type 3 from Afghanistan. In 2000, wild polioviruses of serotypes 1 and 3 continued to be isolated from samples from Pakistan and Afghanistan.

Data on the genetic characteristics of wild viruses from Pakistan and Afghanistan are being made available for use in the poliomyelitis eradication programme through collaboration between scientists at the National Institutes of Health and the Centers for Disease Control and Prevention. There has been a decline in genetic diversity of wild viruses isolated over the years, which supports the evidence of progress towards eradication based on the reduction in the annual number of reported poliomyelitis cases in Pakistan. The number of reported cases was 1147 in 1997, 351 in 1998, 507 in 1999 and 20 up to April 2000. Reported cases include those confirmed on both virological and clinical findings.

Data were presented describing some of the achievements of the poliomyelitis eradication programme in Pakistan. There have been improvements in AFP surveillance, reflected in increases in the reported annual non-polio AFP detection rate in children less than 15 years, which was 0.72 in 1997, 0.69 in 1998 and 1.22 in 1999. There has been a concomitant decline in the number of confirmed poliomyelitis cases in Pakistan in the same period. One factor that contributed to the improvements in AFP surveillance has probably been the use of Special Teams On Polio eradication (STOP) teams, who have conducted training at provincial and district levels, critically reviewed and analysed reported surveillance data and assisted in microplanning for supplementary immunization activities. Only one NID was carried out in 1997 but since that time the number of supplementary immunization activities has increased. Since 1998, cross border immunization campaigns were conducted in co-ordination with the neighbouring countries of Afghanistan and the Islamic Republic of Iran. Since 1999 the number of NIDs per year has been increased and there is greater use of house-to-house immunization approaches to increase the NID coverage. As a result of improvements in surveillance and immunization, some polio-free geographical areas appear to be emerging in Pakistan in Baluchistan, Sindh, Federally Administered Tribal Areas and Azad

Kashmir provinces. The significant progress that is being witnessed in Pakistan would not have been possible without strong government commitment, financial and technical support of various international partners, the motivation of the staff of the national expanded programme on immunization (EPI) and the implementation of extra activities to accelerate progress towards the achievement of the eventual goal of poliomyelitis eradication.

3.2 Egypt

Ms Iman Mohamed Al Maamoun, VACSERA, Egypt

VACSERA serves as a national poliovirus laboratory for Egypt. It also performs primary virus isolation from faecal samples referred from several other countries and intratypic differentiation of polioviruses referred from other WHO network laboratories. In 1999 samples from the countries of Cyprus, Iraq, Jordan, Lebanon, Qatar, Sudan and Syrian Arab Republic accounted for 32% of poliomyelitis related activities done at VACSERA. The laboratory also serves as a training centre for scientists from WHO network laboratories and distributes cell lines for use in these laboratories. VACSERA collaborates with specialized poliovirus laboratories in the United States and the Netherlands to determine the genetic characteristics of the wild poliovirus isolates.

In 1999 VACSERA tested faecal samples from 288 AFP cases from Egypt. Seventy-nine per cent of the AFP cases had adequate stools. Of the 288 cases: 214 had no viruses isolated, 55 had NPEV, 10 had only Sabin-like polioviruses and 9 had wild polioviruses. The wild virus cases were detected in the southern governorates of Assiut (1 of serotype 1 and 1 of serotype 3), Al Minya (1 of serotype 1 and 1 of serotype 3); Sohag (2); Menoufia (1); Qalubia (1); and Benisuef (1). All cases were less than 13 months old and were detected in the low season for virus transmission. The recorded immunization history for the wild virus cases was as follows: 6 received more than 3 doses of OPV, 1 case received only 1 dose of OPV and the immunization history was unknown for 2 cases. Poliovirus serotype 2 has not been detected in Egypt since 1994.

The genetic characteristics have been determined for all wild viruses isolated from Egypt since 1996. All viruses detected in 1999 were indigenous to Egypt. The polio 1 viruses represented continuation of transmission of an indigenous Egyptian genotype designated NEAF1. However, it was of great concern that the closest genetic relative of the polio type 3 virus detected in Assiut in February 1999 was a virus that was last detected in Al Minya in June 1996. The second polio 3 case in 1999 was from Al Minya, from a case with disease onset in November 1999, and its closest relative was a virus isolated from a case in Qena in December 1996. The analysis of the type 3 viruses indicates that 2 separate genetic lineages were transmitted for approximately 30 months without detection through the routine surveillance system. This information has been used to target immunization and improve surveillance activities in the identified high-risk communities.

Egypt has made tremendous progress towards achieving the goal of poliomyelitis eradication, and impressive performances have been achieved in both surveillance and immunization. The number of reported wild poliovirus cases in Egypt has declined over the years and in the most recent 4 years the total cases reported were 101 in 1996, 14 in 1997, 35 in 1998 and 9 in 1999. Between January and April 2000, there were 77 AFP cases from Egypt with samples referred to VACSERA. Two wild virus cases were detected in Assiut in 2000; a

type 1 case with disease onset in January and a type 3 case with onset in February. Consideration is being given to implementing an environmental surveillance project, in addition to the existing AFP surveillance, to try to better define the communities with silent wild virus transmission.

3.3 Sudan

Mr Hatim Osman, National Poliovirus Laboratory, Sudan

The national poliovirus laboratory (NPL) in Sudan tested 148 specimens in 1999, comprising 131 specimens from AFP cases and 17 from contacts. Polioviruses were isolated form 14 cases. Two cases had only Sabin-like viruses, and 12 had wild viruses. Only 8 of the cases had disease onset in 1999; 7 were of serotype 1 and 1 was of serotype 3. A second wild virus serotype 3 case was detected in the southern part of Sudan in 1999, but primary testing was done at the NPL in Kenya and intratypic differentiation at the RRL in South Africa because of more convenient transport links for samples referral. Between January and April 2000 the NPL in Sudan tested 100 specimens and isolated wild polioviruses of serotype 1 from 6 specimens from 3 AFP cases.

The NPL has shown steady improvement in its performance. In 1997 and 1998, the NPL was not accredited by WHO and several problems were detected with respect to technical performance and provision of laboratory supplies. These weaknesses have been addressed and extensive staff re-training done. Consequently the NPL was provisionally accredited in 1999 and fully accredited in 2000.

The laboratory infrastructure is also being upgraded and the NPL is due to be relocated to a recently renovated building. Many new items of equipment have been purchased for the NPL. However, 2 freezers and an electricity generator are still needed for the new facility. The NPL recently received the sum of US$ 5000 from WHO/HQ for procurement of a new computer and printer and for installation of e-mail. This is a welcome development since the NPL has had a computerized database for over 2 years but did not previously have its own printer or direct e-mail access. There is high motivation and commitment of the laboratory staff to the programme of poliomyelitis eradication.

3.4 The Islamic Republic of Iran

Dr Rakshandeh Nategh, National Poliovirus Laboratory, Islamic Republic of Iran

In 1999, the NPL in the Islamic Republic of Iran investigated a total of 854 specimens of which 553 were from 285 AFP cases and 301 were from contacts. Fifty-six per cent of AFP cases were male and 44% were female. There is excellent performance in the Islamic Republic of Iran for detection of AFP cases. There are 28 reporting districts in the country and 70% of them achieved or exceeded the expected number of AFP cases for 1999. Nationally, 78% of the expected number of AFP cases was detected in 1999. Stool samples were collected within 14 days of paralysis onset for 83% of all reported AFP cases. Ninety four per cent of AFP cases had 2 stool samples referred to the NPL, and, 53% of all faecal samples were received in the laboratory within 3 days of collection. Laboratory results were reported within 28 days for 89% of specimens from AFP cases and 88% of contacts.

Results of testing in 1999 showed that: no viruses were isolated from 250 (88%) AFP cases; 27 cases had NPEV isolates; and polioviruses were isolated from 8 cases. Of the latter, 5 had Sabin-like viruses and 3 had wild poliovirus serotype 1 viruses. Polioviruses were also isolated from 34 of 301 investigated contacts, but all of these isolates were Sabin-like.

Epidemiology data on the 3 wild virus cases in 1999 suggested that 2 were imported cases as they had disease onset in Afghanistan. The third case was probably exposed through Afghan visitors to the home of the patient who had not been immunized. Immunization had been in fact been refused for the child in the past when offered by health authorities. Genetic data on the virus isolated from this child did not exclude the possibility that the virus was imported from Afghanistan. However the genetic evidence was not as strong as that obtained from analysing the viruses from the 2 cases which had disease onset in Afghanistan, for which > 98% genetic sequence similarity was found to contemporary viruses isolated from cases detected in Afghanistan.

Concern was expressed about the high prevalence of residual paralysis found in AFP cases at 60 days follow-up in the Islamic Republic of Iran, in the absence of isolation of polioviruses. Of the 285 cases in 1999, it was reported that 83 (29%) had residual paralysis and 8 (2%) had died by the 60-day follow-up visit.

4. MOLECULAR EPIDEMIOLOGY OF POLIOVIRUS

4.1 Role of molecular sequencing in poliovirus eradication: use in different epidemiological situations

Dr Mark Pallansch, Centers for Disease Control and Prevention, Atlanta, USA

Virology laboratories can assist in providing data to answer several basic surveillance questions frequently asked in the poliovirus eradication programme. Data can be obtained to describe the occurrence of particular poliovirus serotypes, the patterns of their transmission, the possible location of reservoirs for wild viruses and the virological indicators of programmatic progress. However, the utility of molecular sequencing data depends on the stage of the eradication programme. In highly endemic countries sequencing of representative isolates is advised to obtain baseline information on the initial biodiversity of viruses. This information can also be used for the development of reagents for use in tests based on genetic characteristics of viruses e.g. probe hybridization and polymerase chain reaction. Isolates obtained from outbreaks in highly endemic countries should be sequenced to determine the source(s) of the outbreak virus. For countries in the intermediate phase of eradication the majority of isolates should be sequenced. These data can assist in identifying the main reservoirs that help to sustain transmission, identify gaps in surveillance and assist in monitoring the genetic diversity of viruses that continue to be transmitted. Investigation of viruses from outbreaks that occur in countries in the intermediate stage can help to distinguish between virus importations and previously undetected indigenous virus. In the final stages of the eradication programme every wild virus should be sequenced to identify remaining reservoirs, dissect paths of transmission, and identify possible contaminant viruses. An important additional activity in the final stages of eradication is to conduct surveillance to detect atypical OPV-derived viruses in polio-free areas. There is a particular interest in the potential for circulation of vaccine-derived viruses.

A decision has been taken in the WHO poliovirus laboratory network that, beginning in 2000, all wild polioviruses will be sequenced. The only exceptions will be viruses from very large outbreaks or highly endemic states or provinces. It has been recommended that the complete VP1 gene should be sequenced and that standard methods of analysis should be applied.

Examples were provided of various applications of molecular sequencing, as follows:

- The finding of identical sequences for viruses from different individuals who have no epidemiological linkage almost always represents laboratory contamination.

- Genetic sequencing can confirm virus identification and provide intratypic differentiation results for problem samples, e.g. mixtures of viruses or atypical viruses that give problematic intratypic differentiation results.

- Genetic sequencing of viruses from Afghanistan, Islamic Republic of Iran and Pakistan has provided several programmatically useful bits of information. There has been a great reduction in the genetic diversity of viruses isolated from Pakistan and Afghanistan. A common polio serotype 1 virus genotype spans these two countries. Viruses detected in the Islamic Republic of Iran in 1999 represented importations from Afghanistan based on both epidemiological and genetic sequencing data. The most recent polio 1 isolates from Pakistan appear to fall into 2 main genetic groups roughly corresponding to geographical distribution in northern and southern parts of the country. However many separate transmission chains still exist in Pakistan and substantial programme action is still needed throughout the country to break these transmission chains.

- Genetic sequencing has been useful to clarify sources of viruses imported into previously polio-free areas. Importation of viruses is facilitated through population movement. Long-range importation is possible across continents or from one to country to another, even if geographical or political borders are not shared. Cross-border transmission is possible among countries that share borders and has been documented among the countries of: Afghanistan, Pakistan and the Islamic Republic of Iran; Turkey and Iraq; and India/Bangladesh/Myanmar.

- Not all parts of an endemic region or country may represent a reservoir for wild viruses. Reservoirs can be considered to be areas in which viruses persist during the low season for transmission and from which viruses are transmitted when conditions are appropriate. Reservoirs can be recognized within an individual country by genetic sequence similarity among isolates from the low season (or beginning of the high season) and those from the same geographical area but detected in the preceding high season. Different reservoirs can also exist in separate countries. For example different reservoirs for type 3 viruses exist in Egypt and Sudan. A type 1 virus reservoir spans the countries of Pakistan and Afghanistan and is distinguishable from a reservoir that spans India, Nepal and Bangladesh.

- Genetic sequence data can be used as indicators of progress. It has been possible to document the disappearance of virus genotypes in several countries. Reduction in

genetic diversity has also been documented among viruses isolated in Pakistan, India and Egypt, even as improvements were made in AFP surveillance and larger numbers of cases were being detected. Further, it has been possible to use sequence data to identify areas within countries in which AFP surveillance may be inadequate, when the isolation of genetically related viruses has been shown to occur although separated by long periods without detection through the routine AFP surveillance system. In Egypt, for example, poliovirus serotype 3 was detected in 1999 after an absence of almost 30 months.

The above examples show that genetic sequencing of wild viruses adds to the surveillance information that can be used for strategic planning and for public health action. Molecular sequencing of polioviruses can provide answers to key surveillance questions and its use and importance increases as the eradication programme progresses. Greater use will be made of this important tool since the capacity for sequencing has been increased within the laboratory network that supports the global poliomyelitis eradication programme.

4.2 Molecular characterization of polioviruses from Iraq

Dr Harrie van der Avoort, RIVM, Netherlands

The transmission of wild polio serotype 1 viruses in Iraq is linked to virus transmission in Turkey, even if these countries are served by different regional offices of WHO. Turkey was the source of 6 of 7 confirmed wild poliovirus cases reported in the WHO European Region (WHO/EURO) in 1997, and of all wild poliovirus cases reported in 1998. The last wild poliovirus detected in WHO/EURO occurred in Turkey and was from a case that had paralysis onset in November 1998.

RIVM has been collaborating with WHO/EURO and WHO/EMRO in the characterization of viruses from different countries, including those from Turkey and Iraq. Viruses that occurred in Iraq in 1994 and the Netherlands in 1992 were genetically related to each other and both probably had ancestral links to viruses from Pakistan. In Iraq, at least 2 separate genetic genotypes of the polio 1 serotype were transmitted in 1994.

Viruses transmitted in Turkey and Iraq since 1995 belong to the same genotype, and these viruses could be distinguished from those that were detected during outbreaks in Finland (1985), Turkey (1990 and 1994), Tajikistan (1991), Ukraine (1993), Uzbekistan (1994), Chechnya (1995), Turkmenistan (1996) and Egypt (1994 to 1996).

Between 1995 and 1999, the total number of wild poliovirus cases reported annually from Iraq and Turkey was as follows:

- Iraq: all wild virus cases since 1995 had serotype 1 isolates and the number reported was 1 in 1995, 0 in 1996, 2 in 1997, 0 in 1998 and 68 in 1999

- Turkey: 1 type 3 in 1995; 0 in 1995, 1996 and 1997; 6 of type 1 in 1998; 27 of type 1 and 3 of type 3 in 1998.

In May 1999 there was great concern about the source of viruses that were detected in 3 AFP cases in Iraq. A 150-nucleotide fragment in the VP1/2A region of the genome was

sequenced and analysed to determine the probable transmission source of viruses from the 3 cases. Statistical analysis showed over a 98% sequence homology between viruses from 2 of the Iraq cases and viruses that had been isolated in Turkey in 1998. The virus from the third Iraq case appeared to be genetically different on initial analysis. It was later found that this virus had a 100% sequence match for the 60 nucleotides from the VP1 gene, but only 80% sequence similarity to Turkish viruses for the 60-nucleotide segment from the VP2A gene. It was deduced that the virus was derived from the same genotype as the Turkish virus but that a recombinant event had occurred in the VP2A gene with an undetermined virus, possibly a NPEV. Ultimately, the genetic sequence of the complete VP1 gene was determined for 28 of the 68 wild virus cases that occurred in the Iraq in 1999 through collaboration between 2 specialized laboratories. It has been determined that 2 genetic lineages of a single polio 1 genotype were transmitted during the outbreak and that this genotype is the same one that has been detected in Iraq and Turkey since 1995. Additionally, sequences corresponding to 60 nucleotides of the VP2 gene were available for 11 viruses and 8 of these viruses were shown to have sequences suggestive of recombination in the VP2A gene. Despite these interesting findings there is no evidence to suggest that these changes have any public health significance. The majority of the cases that occurred in Iraq in 1999 were either unimmunized or incompletely immunized because they received less than 3 doses of OPV. The implementation of NIDs, with use of a house-to-house immunization strategy in most areas, stopped the outbreak and only 4 wild virus cases were confirmed in Iraq between January and April 2000.

5. LABORATORY QUALITY ASSURANCE

5.1 Proficiency test performance in 1999

Dr Harrie Van der Avoort, RIVM, Netherlands

The annual proficiency test provides an opportunity to evaluate the accuracy of laboratories and their adherence to WHO recommended procedures for virus isolation and characterization, and to determine how testing reagents perform in field conditions. Some changes have been made in recommended procedures since the laboratory network was first established. In the past, laboratories were required to use the Hep-2 and RD cell lines for virus isolation and to serotype polioviruses using antisera provided by WHO, and NPEV using antisera provided by RIVM. Current practice is to use the L20B and RD cell lines for virus isolation and to serotype polioviruses using antisera provided by WHO; there is no longer a requirement for serotyping of NPEV. The flow chart for use of L20B and RD cell lines was reviewed.

The nature of samples distributed for proficiency tests has changed because the responsible specialized laboratory no longer distributes wild viruses to remove the potential for contamination of routine work in laboratories with wild viruses of extraneous origin, and to avoid unnecessary, expensive and time consuming field responses. It has not been possible to prepare a single kind of non-wild virus test material that can be used to evaluate all WHO recommended procedures for virus isolation and intratypic differentiation. Consequently only two kinds of proficiency test panels were distributed in 1999. These have been tested and found to be of low risk, good quality and stable during transport in field conditions. A panel of 5 samples containing only Sabin-like polioviruses and NPEV was distributed to evaluate proficiency in virus isolation and serotyping. A panel of 18 inactivated polioviruses was

distributed to evaluate proficiency in intratypic differentiation using the ELISA test. Studies are continuing to identify suitable materials to test proficiency in the probe hybridization, PCR, RFLP and monoclonal antibody neutralization methods for intratypic differentiation.

A description was given of the scoring system used for evaluating proficiency in virus isolation and serotyping. Some features included high penalties for failure to isolate polioviruses or for contaminating samples during testing, and no points are assigned for NPEV serotyping. In 1999, 7 laboratories in the WHO Eastern Mediterranean Region received a panel of 5 samples to test proficiency in virus isolation, and were assigned scores of: 100% (3), 85% (1), 80% (2) and 60%(1). Feedback was provided to the latter laboratory on areas of weak performance, and a new panel was sent for evaluation, for which the laboratory was subsequently assigned a score of 100%. Five laboratories received a panel of 18 samples to evaluate proficiency in ITD by ELISA and scores were 100% (4) and 97%.

5.2 Changes to the accreditation process

Dr Raymond Sanders, WHO/HQ

The accreditation process has been one factor influencing improvement in laboratory performance. Accreditation ensures that agreed upon standards are met for technical performance and that annual evaluations are made of the availability of sufficient personnel, space, equipment and reagents to handle the testing workload. The greater reliance on virology results in the final stages of the poliomyelitis eradication programme has necessitated changes in the accreditation criteria. The changes can be summarized as: greater emphasis on decreasing the time taken for referring known poliovirus isolates to reference laboratories to determine whether they represent wild viruses; implementation of quality control procedures for cell lines used for virus isolation; improving efficiency; increasing accuracy; and discontinuing the use of an NPEV isolation rate of at least 10% as one of the criteria for accreditation. Accreditation criteria are now established for 3 categories of laboratories: national laboratories which perform only virus isolation and serotyping; national laboratories that also have the capability to perform intratypic differentiation; and regional reference laboratories that serve many countries and have capability for virus isolation, serotyping and intratypic differentiation. The designation of "provisional accreditation" has become formally recognized and will apply to laboratories that pass the annual proficiency test but show one or more deficiencies in some other important area that can adversely impact the poliomyelitis eradication programme e.g. not providing results on time. Criteria to be fulfilled by each category of laboratory for full accreditation are as follows:

- National laboratories should fulfil the following criteria: minimum workload of 150 samples in 12 months; reporting of results within 28 days for at least 80% of samples from AFP cases; score of $\geq$ 80% for the annual proficiency test; $\geq$ 90% accuracy for routine poliovirus isolation and serotyping by comparison to results obtained in reference laboratories for referred samples; score of $\geq$ 80% for on-site review of laboratory procedures; implementing of procedures for routine quality control of cell lines used for virus isolation; and referring of $\geq$ 80% of poliovirus isolates to reference laboratories within 14 days of detection for characterization as wild or vaccine-like.

- National laboratories performing ITD, in addition to meeting the accreditation criteria established for national laboratories, as of January 2000 must meet the following four

criteria: attaining a score at least 90% in the annual ITD proficiency test; reporting ITD results within 28 days of virus typing for at least 80% of poliovirus isolates; score of ≥ 90 % for accuracy of poliovirus identification by comparison to results obtained in reference laboratories; and score of ≥ 90% for on-site review of laboratory procedures. For evaluation of accuracy in ITD, the laboratory must refer at least 10 poliovirus isolates annually to a reference laboratory, each within 1 month of characterization, so that action can be rapidly taken if problems are detected.

- Regional reference laboratories are required to fulfil "4 + 2" criteria for full accreditation. The four criteria are: attaining a score of ≥ 90% on the annual isolation and serotyping proficiency test; attaining a score of ≥ 90% on the annual ITD proficiency test; reporting ITD results within 28 days of sample receipt for at least 80% of poliovirus isolates; and attaining a score of ≥ 90% for on-site review of laboratory procedures. An additional two criteria must be fulfilled by reference laboratories that also perform primary virus isolation from AFP cases. These criteria are: reporting results within 28 days for at least 80% of samples from AFP cases; and implementing procedures for routine quality control of cell lines.

5.3 Current accreditation status of poliomyelitis laboratories

Dr Esther de Gourville, WHO/EMRO

Nine of the 12 network laboratories were fully accredited in 1999 (Egypt, Islamic Republic of Iran, Jordan, Kuwait, Oman, Pakistan, Saudi Arabia, Syrian Arab Republic and Tunisia) and 3 were provisionally accredited (Iraq, Morocco and Sudan). In Iraq and Morocco the only deficiency in 1999 was failure to provide timely results. In Sudan the deficiencies were delays in providing results and receipt of less than 150 samples for analysis. As of 30 April 2000, accreditation visits were made to laboratories in Morocco, Oman, Pakistan and Sudan. Morocco remained provisionally accredited and the other 3 laboratories were fully accredited. Morocco again showed poor performance with providing results on time.

It is anticipated that several of the laboratories will have problems meeting the newly introduced criteria for referring polioviruses to reference laboratories for testing within 14 days of detection. An in-depth review was made of the reported laboratory data for 1999. Five of 12 laboratories had no need to refer viruses to another facility because they have in-house capability to perform ITD. However polioviruses were referred when they were detected in laboratories in Iraq, Jordan, Morocco, Oman, Saudi Arabia, Sudan and Syrian Arab Republic. The mean difference between the date of detecting a poliovirus and the date of receipt of the isolate in a reference laboratory was: 26 days for Iraq; 234 days for Jordan; 105 days for Morocco; 24 days for Oman; 33 days for Saudi Arabia; 24 days for Sudan and 64 days for the Syrian Arab Republic. In the past, a variety of reasons were given for delays in sample referral, including the time taken for making arrangements with commercial couriers, lack of funds for paying couriers, lack of appropriate packaging materials, refusal of shipment by commercial couriers and delays in obtaining approval for shipping from the Ministry of Health. Occasionally, some laboratories have sent the original faecal sample, causing further delay as viruses had to be re-isolated for testing in the reference laboratory. The WHO Regional Office is working with individual laboratories to resolve sample referral problems to minimize delays in confirmation of wild poliovirus transmission. This is of critical

importance for the implementation of field activities to minimize transmission, especially as countries approach the final stages of poliomyelitis eradication.

5.4 Common problems and solutions in cell culture

Dr Glyn Stacey, National Institute for Biological Standards and Control (NIBSC), United Kingdom

The identification of polioviruses in patient samples relies on virus isolation in tissue culture cell lines. Problems with cell cultures can lead to inaccuracies in laboratory results. Various measures can be used to determine cell line purity and freedom of cells from infection with microbial contaminants. Cell lines should be obtained from reputable sources. Specialized laboratories can determine the authenticity of cell lines by examining a number of cellular characteristics, using karyology, DNA analysis, isoenzyme analysis, and histological and immunological markers. Users should be familiar with the characteristic morphology of the cells and their specificity for virus isolation. Some simple measures can be taken to maintain the purity of cell lines in routine use, as follows:

- Cell lines should be manipulated in a bio-safety cabinet, using appropriate aseptic techniques to avoid introducing contaminants from the environment. Only one cell line should be handled at any given time, to avoid cross contamination among different cell lines.

- New cells received from another facility should be quarantined in the laboratory until they are shown to be free of contaminants. Quarantine may include the use of separate facilities and reagents to those used for the routine work, or chronological quarantine so that new cells are handled at the end of the working day.

- Cells should be grown in the absence of antibiotics for rapid detection of bacteria and yeast contaminants. Contaminated cultures should be discarded, as attempts to clean of cure them are usually futile. Special measures should be taken to avoid *Mycoplasma* contamination, as these organisms can infect without causing overt cellular changes, while reducing the cell line sensitivity for virus isolation. Cell lines should be either evaluated for the presence of *Mycoplasma* or obtained only from reputable suppliers who document both the absence of *Mycoplasma* and the testing methods used. Prolonged subculture of cell lines beyond 3 months should be avoided, as this can sometimes decrease cell line sensitivity for virus isolation and/or increase the potential for *Mycoplasma* contamination.

- Routine testing for sterility of cell cultures and culture reagents should be implemented as well as quality control of stocks in cell banks.

- Particular attention should be given to regular general housekeeping to prevent the build-up of contaminants in the laboratory environment. Water baths, sinks and other damp environments may promote the growth of yeast and bacteria.

5.5 Quality control of cell cultures

Dr Mark Pallansch, Centers for Disease Control and Prevention, USA

Cell cultures should be shown to replicate and grow with stability and to have a high, reproducible sensitivity for virus growth. Special quality control measures are required because sensitivity for virus isolation is not correlated with any visible cellular changes, and routine subculture of cell lines affects sensitivity in unpredictable ways. Additionally, contamination of cell cultures can change their viability and sensitivity. Cell cultures should be replaced with new stocks from liquid nitrogen every 3 months to avoid some of these problems. However, periodic testing of cell cultures for sensitivity to virus growth should be done routinely every 1 or 2 months and whenever major cell culture media components are changed.

A standard protocol is being developed for use within the global poliovirus laboratory network for quality control of cell lines. The protocol involves the growth of a large batch of Sabin poliovirus serotype 2 to serve as an in-house quality control standard. The material should be subdivided into a large number of single use aliquots to be stored frozen for use as needed. This material should be calibrated against an international standard to determine the virus titre and its reproducibility. The titre of the international standard and the prepared in-house standard must be shown to be reproducible when tested in parallel at least 3 times before the in-house control is deemed ready for routine use. Once this has been done an aliquot of the in-house control can be removed and tested whenever the quality control of the cell lines is to be done. The quality control test will involve testing standard dilutions of the control material in the cell line of interest, observing for 5 to 7 days, and determining the titre compared to the established reference value. No decline in sensitivity can be deduced if the measured titre is the same (± 0.3 log10). Evidence of decline in sensitivity should result in repeating of the quality control test, and replacing cell lines if reproducible results are obtained. Cell line sensitivity for virus growth is vitally important for successful laboratory results. Periodic testing of cell culture sensitivity should be a component of quality control and a simple protocol can be used for sensitivity testing.

5.6 Description of the revised poliovirus laboratory manual

Dr Raymond Sanders, WHO/HQ

An updated manual of procedures used in the global poliovirus laboratory network is being prepared, the format of which will be different to the previous manual. The proposed changes will make the manual more appropriate to current activities, more consistent with the accreditation process and having a greater emphasis on requirements for good laboratory practices. A modular format will be used and the manual will be accessible by Internet. It is envisaged that it will be easy to update sections of the manual as needed. An English version will be produced and consideration will be given to producing the manual in other languages. The proposed layout of the manual will include the following modules:

- the role of laboratories in the programme of poliomyelitis eradication
- good laboratory practice
- specimen and isolate transport
- tissue culture

- specimen receipt and processing
- virus isolation and typing
- intratypic differentiation.

Each section will contain tips on good laboratory management, data requirements, performance requirements and trouble-shooting. It is anticipated that a draft copy of the revised manual will be available by 30 June 2000 and that the final version will be adopted at the Global Polio Laboratory Network meeting in Geneva in October 2000. A published hard copy of the manual should be available by January 2001.

5.7 Recommended methods for intratypic differentiation

Dr Raymond Sanders, WHO/HQ

Recommendations were made on intratypic differentiation (ITD) methods at the WHO Global Polio Laboratory Network meeting in Geneva in 1999, and are as follows:

- ITD should be performed on all poliovirus isolates using 2 methods chosen from:
  - ELISA (RIVM method)
  - Nucleic acid probe hybridization (CDC method)
  - Diagnostic PCR (CDC method)
  - PCR/RFLP (Institut Pasteur method)
- Alternatively, ITD should be performed using any one method chosen from the previous list, if genomic sequencing is carried out on all suspected wild isolates.

A promising technique has been described in which monoclonal antibodies can be used in a polio neutralization test for ITD (MoAB/Neut test). This technique is the product of collaborative research between scientists at the Institut Pasteur, France, and, the National Institute for Biological Standards and Control (NIBSC), United Kingdom. It is anticipated that there will be a formal validation of the protocol for the MoAB/Neut test by October 2000 and that arrangements will be made for bulk production of reagents. Introduction of the method will require training of personnel, development of guidelines for troubleshooting and interpreting the results.

Concern has been expressed about the lack of suitable non-infectious materials to be used as controls in some of the ITD methods and as materials for preparation of proficiency test panels. Suitable inactivated positive and negative virus controls and proficiency test materials have been developed for the ELISA, and similar materials are already being prepared for use in the probe hybridization and diagnostic PCR. Inactivated materials for use in the PCR/RFLP and MoAB/Neut tests have not yet been identified.

6. SUPPLEMENTARY SURVEILLANCE FOR WILD POLIOVIRUSES

6.1 General

Surveillance for cases of acute flaccid paralysis is the primary method of surveillance adopted in the WHO Eastern Mediterranean Region to provide data for the poliomyelitis eradication initiative. However, many countries have conducted other surveillance activities that may be added to their AFP surveillance data for documentation of their achievements and certification of poliomyelitis eradication. The experiences in Tunisia, Netherlands, Morocco and Finland were presented to emphasize the scientific basis, advantages and disadvantages of using surveillance data from non-AFP sources as evidence of the absence of circulating wild polioviruses.

6.2 Investigation of samples from non-AFP patients in Tunisia

Dr Hinda Triki, Institut Pasteur, Tunisia

The virology laboratory at the Institut Pasteur in Tunisia has available data from testing faecal samples of AFP cases, contacts of AFP cases, cases of meningitis and non-AFP paralytic patients. The majority of samples from non-AFP sources were received for routine diagnostic or research work of the Institut, not from activities specifically targeted at poliomyelitis eradication. The laboratory has continued to test contacts of AFP cases, despite WHO's recommendation to the contrary, because the last wild virus in Tunisia was detected in 1994 in a contact. Indeed, the majority of wild polioviruses detected in Tunisia between 1991 and 1994 came from contacts and not AFP cases.

Between 1995 and 1999, samples from 175 paralytic non-AFP cases were analysed. These cases were originally categorized as AFP and their samples were received and processed for analysis. Subsequently the cases were discarded as AFP because clinical review deemed that they did not meet the case definition. Nevertheless virology results are available for these non-AFP cases and 1% of 175 samples yielded Sabin-like polioviruses.

In 1997 there were 173 reported cases of aseptic meningitis in 2 governorates of central Tunisia with 4.6 % case fatality. Faecal and cerebrospinal fluid (CSF) samples from these cases were investigated. All faecal samples were negative for enteroviruses, but West Nile Fever (WNF) virus was identified by serology in some CSF samples. West Nile Fever RNA was also detected in necropsy specimens from 1 case. Subsequently at least 5 micro-epidemics of aseptic meningitis occurred in Tunisia between 1998 and 1999, and virology investigations identified Echo 11, Coxsackie B virus and Echo 1 viruses as causative agents of 3 epidemics (work of Bizerte in 1998, Gabes in 1999 and Tatouine in 1999).

At the Institut Pasteur in Tunisia the routine procedure for investigating suspected cases of aseptic meningitis or micro-epidemics involves inoculation of CSF and stool samples into RD, L20B, Hep-2 and MRC-5 cell lines, and use of PCR to detect enteroviruses. If negative results are obtained, investigations proceed to look for arboviruses or other viruses. Sporadic cases of meningitis are similarly investigated, but the range of investigations is extended to include Herpetoviridae by using PCR for detection of cytomegalovirus, herpes virus and Epstein Barr virus.

6.3 Enterovirus surveillance in the Netherlands

Dr Harrie Van der Avoort, RIVM, Netherlands

A number of surveillance activities are carried out in the Netherlands that would allow detection of wild polioviruses. The scientific basis for the absence of wild poliovirus in the Netherlands is the evidence obtained through:

- obligatory notification of suspected poliomyelitis cases;
- routine AFP surveillance through which approximately 30 AFP cases were reported per year using a passive reporting system;
- environmental surveillance in high-risk areas in which populations reside who refuse polio vaccination. Specifically, 8 sewage water samples are tested per month from secondary schools attended by a high percentage of non-vaccinees; and
- laboratory-based enterovirus surveillance.

Laboratory based enterovirus surveillance is ongoing, and an evaluation has been made of the quality of the evidence from this activity for documentation of the absence of wild poliovirus circulation. Enterovirus surveillance is advantageous as it includes patients, samples and methods that are relevant to the poliomyelitis eradication programme. However, it also extends surveillance to include other patients or samples from the population. In the Netherlands it is based on an existing infrastructure so there is no need to develop a separate surveillance activity restricted only to poliomyelitis. Disadvantages are that the sensitivity of enterovirus surveillance for detecting poliovirus is unclear and no guidelines or performance indicators have been developed. The enterovirus surveillance system in the Netherlands depends on the collection of reports of routine diagnostic investigations performed in a network of clinical virology laboratories. Two categories of reports are received:

Twenty regional laboratories that have routine quality control procedures in place and participate in a proficiency test scheme report weekly. Reports include positive diagnoses for all viruses and samples types. Data are confined to only the results of the reporting week and no corrections are made to previous reports. There are some disadvantages to these reports for surveillance for poliomyelitis eradication: serology results are included with results of analysis of throat swabs and CSF, which are not the recommended techniques or samples for detecting polioviruses; no data are provided on patients or negative samples; and polioviruses may be missed if typing is not done in the same week of the report, enteroviruses are not typed or untypable enteroviruses are not subjected to additional tests.

Twenty regional laboratories report quarterly and provide information suitable for surveillance for poliomyelitis eradication. Reporting laboratories do a proficiency test for polio and enterovirus detection and typing and must exclude the presence of polioviruses in all untyped and untypable viruses by growing them in L20B cells. Quarterly reports provide only the results from samples analysed on polio sensitive cells, positive and negative results for stool samples, age of patients and results of serotyping of viruses.

In addition to the above, all polioviruses and a selection of NPEV are collected from reporting laboratories for further analysis by an accredited national poliomyelitis reference laboratory. There is rapid ITD and molecular characterization of all polioviruses, and tests are performed to confirm the absence of polioviruses in untypable NPEV and a selection of typed NPEV. These measures allow an assessment of the quality of the isolation and typing of enteroviruses in the network of laboratories in the Netherlands. Laboratory based enterovirus surveillance has provided data about the number of enterovirus infections notified in the Netherlands between 1995 and April 2000. Data for the period 1996 to 1998 showed that 19 240 samples were tested, of which 15 168 were from persons younger than 15 years of age and 4072 from persons over 15 years of age. Enteroviruses were isolated from 5.2% of all samples; 6.4% of samples from patients less than 15 years and 1.1% from patients more than 15 years old. Only 5 polio positive samples were detected: all were from patients under 15 years old, and all were Sabin-like viruses. During 1996 and 1998 polioviruses were excluded from 19 118 (99%) of the 19 240 samples: 18 229 had no viruses isolated; 852 had only NPEV by serotyping of virus isolates; 37 were polio negative because they were NPEV positive but negative when cultured in L20B cells. The presence of polioviruses was not excluded for 122 (1%) of samples tested between 1996 and 1998.

Many poliomyelitis non-endemic industrialized countries, particularly in Western Europe, are unable to implement adequate AFP surveillance. Data from alternative surveillance strategies are being proposed by such countries in national certification documents. The Netherlands has presented a combination of passive surveillance for suspected poliomyelitis cases and enterovirus surveillance as evidence for absence of wild poliovirus circulation. However, guidelines and criteria for enterovirus surveillance data have not yet been developed nor has the sensitivity been compared to AFP surveillance.

6.4 Challenges of implementing environmental surveillance

Dr Tapani Hovi, National Public Health Institute, Helsinki, Finland

All poliovirus infected individuals, whether presenting with paralytic symptoms or not, excrete infectious poliovirus into the faeces for several weeks, and consequently polioviruses can be released into the environment. In theory, direct searching for circulating poliovirus in the population by analysing environmental specimens could be at least as sensitive as AFP surveillance in detecting the virus. In populations served by a sewerage system, virological analysis of sewage samples has been successfully used to monitor poliovirus circulation in the population. There are also examples from developing countries demonstrating wild type poliovirus in environmental specimens in the absence of reported cases of acute flaccid paralysis (AFP).

An example was presented of the use of environmental surveillance in investigating an outbreak of poliomyelitis in Finland. In the summer of 1984, poliomyelits was diagnosed in a patient by serology, and poliovirus serotype 3 was subsequently isolated from several contacts of the case. It should be noted that Finland used only inactivated poliovirus vaccine (IPV) in its immunization programme and that IPV is not excreted in the stool of vaccines. Therefore such clustering of persons with poliovirus isolates could not have arisen as a result of routine immunization. Screening of sewage samples was intitiated after the detection of polio 3 viruses in the contacts, and viruses were subsequently detected in 13 locations in Helsinki and in 13 other cities or towns all over the country. During the outbreak 10 paralytic cases were

reported in a country of 5 million people, although it was estimated that at least 100 000 people were infected during the outbreak. The outbreak was controlled by mass vaccination with oral poliovaccine, and sewage sampling was used as a tool to evaluate the efficacy of the nationwide campaign. Only OPV strains were detectable in sewage for up to 2 months after the campaign. After the outbreak the country continued to use IPV in the routine immunization programme. The success of environmental sampling for detection of and monitoring of polioviruses in Finland is possible because the population is served by a well developed sewerage system, making possible the collection of samples and esitimation of their representativeness according to population size.

Several technical and operational restrictions, however, limit the usefulness of environmental surveillance in most places. At its best, it can be considered as a targeted tool potentially supplementing the fundamental knowledge based on AFP surveillance. Potential situations to consider for implementation of environmental surveillance include populations with known unrepairable defects in AFP surveillance, relatively small populations where the background AFP incidence (1:100 000) is an inaccurate measure, and other possible reasons to suspect an unidentified reservoir of wild type poliovirus.

Successful implementation of environmental surveillance is possible only if:

- possibilities and limitations of the approach are clear to decision makers and operators
- sampling sites are selected to reliably represent the target populations
- sampling principles, sample transport and laboratory analysis are well planned, taking into account local conditions and capacities of the primary laboratory
- sampling is carried out for an extended period covering the possible peak season
- potential poliovirus isolates are rapidly referred for intratypic differentiation, and any wild type strains for further genetic characterization
- laboratory results on environmental specimens are rapidly reported
- options for response to possible wild type poliovirus isolation in the environment are discussed in advance.

At the moment, cell culture isolation of polioviruses from environmental specimens is feasible in the L20B cells which are not susceptible to most non-polio enteroviruses (NPEV). Parallel use of RD cells, which are susceptible to NPEV could be one potentially useful internal control. OPV-derived viruses, if present in abundance, may prevent detection of small amounts of wild-type poliovirus in environmental specimens. Therefore, the sampling plan should acknowledge that the likelihood of detecting wild type poliovirus is decreased during and immediately after OPV campaigns.

6.5 Environmental surveillance in Morocco

Ms Amal Barakat, Institut National D'Hygiene, Morocco

Two studies on environmental samples have been carried out at the Institut National D'Hygiene, Morocco, as follows:

- One study that was done, in collaboration with researchers at Institut Pasteur, France, compared the impact on the isolation of enteroviruses using two different concentration methods for pre-treatment of water samples of diverse origin collected between 24 March and 10 July 1998. The concentration methods used were glass wool adsorption and glass powder adsorption. Water samples were obtained from the sea and from treated and untreated well water. Sample processing by both concentration methods involved sample adsorption, elution, treatment of eluates with antibiotics, and inoculation onto Hep-2, L20B and RD cell lines. It was found that a higher percentage of isolates was obtained by glass powder adsorption than by glass wool adsorption. Isolates were obtained on RD and Hep-2 cell lines and have been referred to Paris for analysis using PCR techniques. Duplicate isolates were typed at the NPL in Morocco and no wild polioviruses were detected.

- In another study, water samples were collected from different abattoirs and processed by a technique learned at the RRL in Kuwait. Details of sample processing were not presented at the meeting. After concentration, samples were inoculated onto L20B and RD cells. Three of the 10 samples yielded virus isolates on RD cells, 2 of which were Echo viruses and 1 of which was an untypable virus.

In the discussion of the presentation it was mentioned that these studies were not conducted with the objective of contributing to the surveillance for poliomyelitis eradication. Additionally, because of the types and sources of samples analysed, the results will not be considered as relevant to the poliomyelitis eradication programme.

7. LABORATORY EQUIPMENT MANAGEMENT

7.1 Equipment performance as an essential component of quality assurance in virology laboratories

Dr Glyn Stacey, NIBSC, United Kingdom

Equipment performance can affect the accuracy of laboratory results, and monitoring of equipment performance must be one of the components of the quality assurance programme. In virology laboratories the concept of quarantine of infectious materials should be kept in mind for equipment placement. Separate areas should be designated for handling non-infectious and infectious materials. If physical separation cannot be achieved then chronological separation of work should be considered with work proceeding during the working day from sterile to contaminated materials. It is also important that there be coordination among workers if several persons work within a single laboratory or use the same equipment. Special attention should be paid to environmental control in virology laboratories, with provision for waste management, routine cleaning and regular cleaning and disinfection of equipment. While routine fumigation and/or air sampling is not necessary, it is important that there be thorough periodic cleaning within laboratories.

It is often useful to have a single person designated to supervise and coordinate activities related to ensuring proper functioning of equipment. That person should maintain records of the monitoring, maintenance and repair of critical items of equipment and be prepared to advise on safety and good laboratory practice. It is of vital importance that care is taken in the selection of appropriate equipment when new items are being procured. Consideration should be given to the equipment design features, what will be required for installation, how the equipment will be operated and how its performance will be evaluated. Only then should conscious decisions be made about selecting an appropriate supplier. Once equipment is delivered it should be checked to ensure that it conforms to the specifications requested and should be installed in an environment that is suitable for its operation. Confirmatory checks should be made to verify the key aspects of performance in the range of conditions in which the equipment is expected to operate in the local setting. Verification of performance should be done in the absence of contributory effects that might be introduced by the laboratory process or method.

Incubators, cold rooms and autoclaves are critical items of equipment in virology laboratories. The power supply to incubators and cold rooms should be monitored, as well as the temperature profiles while they are in use, and whether a stable temperature is maintained.

Autoclaves should have temperature, timer and chart recorder calibrated, and should have safety checks performed. Micropipettes are often used in inoculation and manipulation of tissue cultures and should be checked for accuracy and precision and calibrated as needed.

7.2 Equipment concerns at the national poliovirus laboratory of Jordan

Ms Karima Howaidy, Jordan

A list was provided of the equipment available at the NPL in Jordan. An electricity generator is available because the NPL is housed within the same facility used for storage of vaccines. There is therefore little disruption in electricity supply to incubators, biological safety cabinets and freezers. The NPL has a contract with a local company called the Royal Scientific Society for maintenance and repair of laboratory equipment. But monitoring of equipment performance is done daily by the staff of the NPL. Critical items, such as refrigerators and freezers, are fitted with temperature sensitive alarms. The staff of the NPL intends to write a standard operating procedures manual that will include methods of equipment operation, validation methods and procedures for cleaning, calibration and maintenance. New equipment is procured either through local tenders or through WHO.

7.3 Equipment concerns at the national poliovirus laboratory of Saudi Arabia

Mr Moghram Al Amry, National Poliovirus Laboratory, Saudi Arabia

The performance of critical items of equipment is monitored at the NPL. There have been three main problems:

- A designated engineering company is responsible for performing repairs on equipment. In 1999, unscheduled repairs were made to a central air conditioning unit without prior consultation with the laboratory staff. This resulted in gross contamination of the laboratory environment with dust, and problems with contamination of cell cultures. This was only resolved when the laboratory was fumigated and filters were replaced in the bio-safety cabinets. It was subsequently agreed with the representatives of the engineering company that prior notice must be given of any intention to perform repairs on equipment at the NPL.

- No specific budget has been allocated to the NPL for payment for equipment repairs or purchase of spare parts. The experience has been that there are sometimes long delays between requesting and receiving funds from the Ministry of Health for equipment repair.

- Some problems have been experienced in obtaining equipment provided by WHO. Customs charges are levied when items are delivered in Saudi Arabia, and the last batch of supplies remained at the airport because of lack of funds to pay customs duties.

7.4 Equipment concerns in the national poliovirus laboratory of the Syrian Arab Republic

Ms Halla Saba, National Poliovirus Laboratory, Syrian Arab Republic

The laboratory was established in 1991 through collaboration between the Ministry of Health and WHO. The NPL became functional in 1993 and WHO provided essential

equipment, freezers incubators, liquid nitrogen freezers and biosafety cabinet. The Ministry of Health provided other equipment. Some problems have been experienced in getting equipment repaired. The NPL has had one biosafety cabinet non-functional for more than 3 years. The cabinet worked for only 30 hours, and all attempts have failed to get the agent for the company to repair the cabinet. Two –70ºC freezers have also been non-functional for some time. A new freezer and biosafety cabinet have been procured for the NPL by WHO. These items have been received in the Syrian Arab Republic but have remained at the airport for more than 3 months because funds are not available to pay customs charges.

8. LABORATORY PERSONNEL MANAGEMENT

8.1 Demonstration of LABIFA software

Dr Suleiman Al Busaidy, National Poliovirus Laboratory, Oman

A training workshop was conducted in Oman in 1999 for personnel at the NPL. The workshop provided participants with hands-on experience with EPI Info software and with the Laboratory Information for Action (LABIFA) software developed for use in the WHO Eastern Mediterranean poliovirus laboratory network. Participants also learned how to perform standard analyses and prepare and send weekly reports by e-mail. The LABIFA software was demonstrated to participants of the meeting.

8.2 Overview of training for personnel in the laboratory network

Dr Esther de Gourville, WHO/EMRO

Various training activities implemented between May 1999 and May 2000 were as follows:

- Meetings of directors of poliomyelitis network laboratories were held in Alexandria, Egypt, in May 1999 and Muscat, Oman, in May 2000. Both meetings provided opportunities for sharing experiences, priority setting and updating participants on laboratory matters of relevance to the global poliomyelitis eradication initiative.

- A training workshop on ITD of polioviruses was held in Kuwait in November 1999. There were 10 participants from the 5 network laboratories that have capability for ITD. The participants were new staff or persons who had not had prior extra-mural training on the ITD methods. Participants received hands-on experience with ELISA, probe hybridization and PCR techniques and lecture sessions covered the theoretical basis of the various tests and provided tips on troubleshooting. Trainers were from CDC, RIVM and WHO/EMRO.

- Approximately 40 staff from 10 of the 12 network laboratories had data management training in their own countries. Resource persons involved in running training workshops were two computer consultants from WHO/EMRO, and the regional laboratory coordinator. Formal training workshops have not been conducted in laboratories in Kuwait and Egypt, although the regional laboratory coordinator visited the Egypt laboratory on several occasions to work with the staff on data management

issues. All network laboratories have computerized databases and access to e-mail for rapid communication on laboratory issues and sharing of laboratory data.

- Approximately 10 people were trained in cell culture techniques by consultant virologists assigned to the poliomyelitis laboratories in Iraq and Sudan in 1999.

There will continue to be a need for training to respond to staff changes or problems with testing procedures. Additionally, training is required to strengthen management within some laboratories. There may also be a need for specific training for maintenance and repair of biosafety cabinets for a few laboratories that may not have access to the relevant expertise locally. A mechanism has yet to be identified for such training. Currently suppliers are contacted to provide maintenance services as the need arises, but suppliers are not always responsive to requests.

9. CONTAINMENT OF WILD POLIOVIRUSES

9.1 Progress towards implementation of the global containment plan for wild polioviruses

Dr Walter Dowdle, Task Force for Child Survival, USA

Containment can be considered to be the other half of the activities needed to achieve poliomyelitis eradication. When the virus has been eradicated from its human host it is likely that laboratories will be the only major remaining sources of wild viruses and other potentially infectious materials. Population immunity can be expected to decrease after eradication is achieved. Following cessation of OPV immunization, it is likely that the chance introduction of wild polioviruses will pose a serious threat to public health and jeopardize the achievements of poliomyelitis eradication. Linking laboratory containment to the progress of the eradication programme is therefore crucial to achieving success. A global action plan has been prepared to achieve containment, and the plan is divided into 3 phases linked to stages of the poliomyelitis eradication programme. Various activities are recommended to be completed pre-eradication (phase 1), post-eradication (phase 2) and post-OPV immunization (phase 3).

In 1998, The Third Global Certification Commission recommended that for regional certification of poliomyelitis eradication, all countries will need to provide evidence that activities described in phase 1 of the Global Action Plan have been completed. The required activities are:

- to conduct a national laboratory survey

- to establish a national inventory of all laboratories where wild polioviruses and potentially infectious materials are stored

- to institute recommended international standards for bio-safety in laboratories handling or storing samples likely to contain polioviruses.

The WHO Global Action Plan for Laboratory Containment of wild polioviruses describes materials that are potentially infectious as throat, faecal and environmental specimens collected at a time and place where poliomyelitis was/is endemic. The plan

emphasizes that such materials may be found in any clinical or biomedical laboratory (i.e. not only the laboratories directly involved in the poliomyelitis eradication programme) and that the risk from such materials is likely to be random and low, but not zero.

A meeting was held in Atlanta, USA, in April 2000 to discuss progress in global containment. Experiences were presented from various WHO regions as follows:

- Regional certification of poliomyelitis eradication is scheduled for October 2000 in the WHO Western Pacific Region. The Region began implementing the containment plan late and visits from the WHO Regional Office to several countries were essential to get national activities started.

- Certification is anticipated in 2002 for the WHO European Region. Major efforts are under way to gain national commitments. A large number of countries (53) and laboratories will pose a special challenge to achieving containment.

- The WHO Region of the Americas was certified as polio-free in 1994. Containment planning is to be completed by all countries by December 2000. National surveys/inventories are to be completed by December 2001. It is anticipated that the USA will pose the greatest logistical challenge.

- In the WHO African, Eastern Mediterranean and South East Asia Regions, the current priority is the interruption of wild poliovirus transmission. However, countries that have already interrupted transmission will be instructed to initiate the containment process.

At the April meeting in Atlanta there was discussion about some of the uncertainties inherent to the containment process. Ultimately it will not be possible to validate the destruction of all infectious materials in every country. Additional mislabelled stocks or unrecognized potentially infectious materials may be missed and present safety risks that may be low, but should not be discounted. Safety risks may vary according to the laboratory procedures that are used. Finally, a risk that cannot be ignored is that of intentional release of wild virus after OPV immunization stops. Despite these uncertainties, it is anticipated that effective containment will be achieved with effort, over time, and with the commitment of all countries involved in the poliomyelitis eradication effort.

9.2 Containment of wild polioviruses in the WHO Eastern Mediterranean Region

Dr Esther de Gourville, WHO/EMRO

There is international recognition that the job of poliomyelitis eradication will not be completed as long as laboratories remain with stocks of materials that have the potential to cause infection and or disease. There will be a need to achieve laboratory containment of all materials that are known to have polioviruses, or that may unknowingly harbour polioviruses, i.e. potentially infectious materials. There is justification for concern about the need for containment because of documented past incidents of laboratory workers being infected as a direct result of their work and/or infection of persons in contact with laboratory workers. There is also the potential for contamination and virus transmission in communities because of materials handled inappropriately in laboratories. When global poliomyelitis eradication is achieved it is proposed that there will be global cessation of immunization. At that time laboratory accidents that may result in infection with polioviruses will represent a serious public health problem threatening the investments in and achievement of global poliomyelitis eradication. Since the majority of poliovirus infections are asymptomatic there is also the potential that silent transmission of the virus may occur without detection for some time.

Practices in several laboratories provide further reasons for concern, among them:

- routine storage of samples and lack of detailed documentation of stored materials;
- ad hoc arrangements for sample storage when there is electricity or equipment failure;
- implementation of collaborative research projects that result in potentially infectious materials being transported from polio-endemic countries to non-endemic countries;
- re-testing of old materials to evaluate new test reagents, or to detect newly described pathogens; and
- ongoing research on polioviruses.

These practices introduce risks for laboratory accidents and inadvertent handling of poliovirus potentially infectious materials.

A plan has been prepared to achieve the containment of wild polioviruses and other potentially infectious materials in the WHO Eastern Mediterranean Region. Laboratories will be required to choose 1 of 3 options for handling of such materials: implementation of procedures to render the materials non-infectious; destruction of materials; or storing and handling materials only in facilities designed to meet international standards for biosafety. The regional plan conforms to the global action plan that was described above and is to be implemented in 3 phases linked to achieving the goals of poliomyelitis eradication. Phase 1 is already being implemented. Phase 2 is to be implemented 1 year after the detection of the last poliovirus case in the world, and will require that laboratories implement their chosen option for containment of materials, with strong emphasis on encouraging laboratories to destroy materials. Phase 3 will be implemented after global cessation of OPV immunization and will require destruction of OPV stocks (including clinical and manufacturing stocks) and

implementation of maximum biosafety standards in any facility that continues to handle polioviruses or potentially infectious materials.

The roles of the WHO Regional Office in the containment process will be: advocacy; obtaining high level political commitment to ensure that the plan is implemented in every country of the Region; and provision of technical assistance to individual countries. Regional guidelines on containment of polioviruses have been prepared and these can be adapted to suit the needs of individual countries. It is anticipated that every country of the Region will have a national plan on containment available by June 2001 and that all 23 countries will have completed national inventories of laboratories by December 2001.

A number of challenges are anticipated as countries implement the containment plan. In some countries, laboratories operate without any legal requirement for registration or mechanism for monitoring their performance (e.g. accreditation or certification bodies). The exact number of biomedical laboratories may be unknown as well as the scope of their work. Further challenges will be identifying mechanisms to ensure that laboratories operate at the required biosafety standards and/or that containment of materials has been achieved. Laboratories operating outside the health sector will have to be assessed in all countries. Therefore cooperation with and evaluation of laboratories operating within ministries of education, defence and others may be required. Multisectoral involvement will be required in implementing the national plans for containment, and a number of suggestions are provided within the regional guidelines to assist countries in achieving such involvement.

9.3 Experiences with implementing phase 1 of the containment plan in Oman

Dr Suleiman Al Busaidy, National Poliovirus Laboratory, Oman

The Minster of Health of Oman has appointed a national coordinator for containment of wild polioviruses. The coordinator is the director of the National Poliovirus Laboratory and. has heightened awareness of containment issues through lectures and discussions with the National Certification Committee; the National Expert Committee that reviews clinical data on AFP cases; and laboratory personnel in different parts of the countries. The WHO regional guidelines on containment have been adapted for use in Oman and a laboratory survey has already been conducted to determine the storage capacity and materials stored in laboratories in Oman. The survey was conducted by sending a standard questionnaire to 132 biomedical laboratories identified in the national containment plan. Responses were received from 97% of laboratories. Approximately one-third (44) of the surveyed laboratories have capability to store frozen materials, 2 have –80ºC freezers and are known to store poliovirus isolates and other potentially infectious materials. Follow-up visits will be paid to laboratories to determine the exact nature of stored materials and to review biosafety arrangements. It is anticipated that phase 1 of the containment plan will be completed by September 2000.

9.4 Experiences with implementing phase 1 of the containment plan in Tunisia

Dr Hinda Triki, Regional Reference Laboratory, Tunisia

A national coordinator for containment has not been identified nor a national plan written. However, the director of the RRL has undertaken a preliminary review of two laboratories because of her awareness of the content and objectives of the global containment plan. At the RRL an inventory has been made of stored wild polioviruses. The inventory uses a line-list format that records the following information for each isolate: labcode, serotype, year of isolation, origin of the sample (AFP case, contact of other), geographical origin, results of each ITD method and results of partial sequencing if performed. At the RRL wild poliovirus strains are kept frozen in a separate, locked box. Representative stains of all wild viruses were sequenced in the VP1/2A region and sequences are available in the RIVM database. The RRL is ready to implement any recommended containment actions on the stored wild viruses. There is also a plan to prepare a line-list of all untypable enteroviruses isolated at the laboratory. All such viruses isolated prior to 1997 have already been inoculated onto L20B cells to exclude the presence of polioviruses. The biosafety arrangements at the RRL are appropriate for phase 1 of the containment plan. The RRL will destroy all wild polioviruses and potentially infectious materials and will not upgrade its biosafety arrangements unless it is designated to serve as a regional interim repository for viruses from other countries.

In Tunisia, an informal enquiry among virology laboratories identified one facility with an enterovirus collection that included polioviruses that had not been differentiated as wild or vaccine-like. Follow-up action with this laboratory in Tunisia will involve collaboration to perform ITD of the poliovirus isolates and inoculation of other isolates into L20B cells to rule out the possibility that they contain any polioviruses.

10. MEASLES LABORATORY ACTIVITIES

10.1 General

Eight of the 12 laboratories involved in the poliomyelitis eradication programme have been designated to provide support to measles elimination or control programmes in their countries. It was advantageous to discuss measles laboratory issues at the meeting to foster better coordination of activities and optimization of the use of resources for measles and poliomyelitis activities.

10.2 Regional progress towards measles elimination

Dr Faten Kamel, WHO/EMRO

The average regional measles immunization coverage rate was 80% in 1999, and an upward trend in coverage has been sustained since 1996. High immunization coverage was reported by 14 countries, namely: Bahrain, Cyprus, Egypt, Islamic Republic of Iran, Kuwait, Libyan Arab Jamahiriya, Morocco, Oman, Palestine, Qatar, Saudi Arabia, Syrian Arab Republic, Tunisia and United Arab Emirates.

During 1999, there were 71 595 measles cases reported in the Region, which is much less than the 88 941 cases reported in 1998. Nevertheless, measles is underreported because it

is still considered to be a mild illness, and many cases do not appear at health facilities. Therefore the reported cases are generally only those admitted to hospitals, and cases seen in primary health care facilities are usually not reported. In addition, in many countries, no reports are obtained from the private sector.

Three planning workshops on measles were held between 1998 and 1999 to review the measles situation in the countries, discuss recent technical developments in measles control/elimination and update national plans. The first workshop was attended by countries that have already reached the poliomyelitis eradication target and have started or are eligible to start measles elimination activities. Participants were from Bahrain, Cyprus, Kuwait, Islamic Republic of Iran, Jordan, Qatar, Morocco, Oman, Palestine and the Palestinian population served by UNRWA, Saudi Arabia, Syrian Arab Republic, Tunisia and United Arab Emirates. The second workshop included the rest of the countries that are planning for acceleration of measles control, and emphasized the need for raising routine measles immunization coverage, establishing measles surveillance and ensuring that immunization activities are carried out in identified high-risk communities. There will be full implementation of measles elimination strategies in all countries of the Region when the poliomyelitis eradication target is achieved.

Mass measles immunization campaigns have been implemented in the following countries involved in measles elimination programmes:

- Jordan and Bahrain implemented campaigns in late 1997 and early 1998, respectively, targeting school-age children, and a second campaign was conducted in 1999 targeting preschool children.

- Kuwait conducted a national immunization campaign in 1994 and a follow-up campaign in November 1998.

- Oman is maintaining measles elimination status, having carried out a mass immunization campaign in 1994, and achieved extremely high immunization coverage subsequently through the routine immunization programme.

- The Syrian Arab Republic and Tunisia conducted immunization catch up campaigns in late 1998.

- Saudi Arabia conducted a catch-up campaign in October 1998 targeting intermediate and secondary school-age children, and followed this up with a second campaign in 1999 targeted at preparatory schoolchildren.

- United Arab Emirates conducted the first phase of a catch-up campaign in November 1998 that targeted children between 9 months and 5 years of age, and a second campaign to cover school-age children was conducted in 1999.

- Qatar conducted a campaign among 4–18 year old children during 1999.

- Egypt is implementing a plan for accelerated measles control and conducted an immunization campaign in high-risk areas in 1998. The campaign reached approximately two million children.

Realizing the importance of the role of the laboratory for measles surveillance in measles elimination programmes, a regional laboratory network for measles diagnosis is being established.

10.3 Progress towards establishment of a regional measles laboratory network

Dr Hinda Triki, Institut Pasteur, Tunisia

Measles is a disease that is difficult to diagnose accurately on the basis of clinical signs because most symptoms are not unique but also occur in other diseases and conditions. Laboratory analysis is required to confirm the clinical diagnosis. However the extent of utilization of laboratories should be appropriate to the endemicity stage for the disease in any given country. In all countries laboratories are useful to monitor the level of protection against the disease and to confirm clinical diagnosis in the early stages of outbreaks when only a few patients need to be tested. Laboratory diagnosis of every case of measles is only required in those countries in a measles elimination programme when transmission is substantially reduced.

A description was provided of the laboratory network that is being established for measles and the scope of work for designated global, regional, national and sub-national laboratories. Three main laboratory procedures will be carried out at various levels in the laboratory network: IgM ELISA, virus isolation and genetic sequencing.

Within the WHO Eastern Mediterranean Region, each country has been requested to identify a national laboratory, and a regional laboratory will be selected. Two intercountry laboratory training workshops have been held at the Institut Pasteur in Tunisia. To date ELISA and virus isolation techniques have been taught to participants from: Bahrain, Egypt, Islamic Republic of Iran, Jordan, Lebanon, Morocco, Oman, Pakistan, Saudi Arabia, Syrian Arab Republic and United Arab Emirates. Each participant received a proficiency test panel for evaluation after the training workshops and all performed satisfactorily. Consultant virologists made on-site visits to national laboratories designated in Bahrain, Egypt, Islamic Republic of Iran, Kuwait, Morocco, Syrian Arab Republic and United Arab Emirates. The visits were to review measles and rubella testing capability, determine resource needs and explore the need for expansion to include sub-national laboratories.

10.4 Measles in Tunisia

Dr Hinda Triki, Institut Pasteur, Tunisia

Measles vaccination was introduced in Tunisia in 1983 and doses of measles vaccine are given at the ages of 9 and 15 months. Vaccination coverage was 65% in 1985 but has been sustained at over 90% since 1992. Up to 1998, surveillance was based on passive reporting of measles cases by clinicians, regardless of whether there was laboratory confirmation of the diagnosis.

Tunisia started a measles elimination programme in 1998. A mass immunization campaign with measles vaccine was carried out in November 1998 targeted at children between 7 and 15 years old. The routine vaccination schedule was changed to 2 doses of measles vaccine given at 16 months and 6 years of age. Measles surveillance activities were strengthened. A new case definition was used and there is reporting of suspected cases. Laboratory confirmation of measles has been introduced. Two laboratories are involved in the programme: one at Charles Niccolle Hospital performs serological assay, and the other, at Institut Pasteur, performs virus isolation and serology. At Institut Pasteur, serological investigations include measles IgM on all samples and rubella IgM on measles IgM negative samples. Between 1997 and 1999 the Institut Pasteur laboratory investigated 188 specimens and found 75 (40%) to be negative for investigated viruses. The remaining samples comprised: 3 measles IgM positive; 91 rubella IgM positive; 1 parvovirus B19 positive; 17 human herpes virus 6 positive; and 1 West Nile virus positive. Therefore rubella appeared to be a larger contributor to febrile rash illness than measles virus during 1997 and 1999 in Tunisia. Rubella vaccination was not included in the expanded programme on immunization (EPI).

Suggestions for how measles surveillance could be further strengthened in Tunisia, include provision of measles IgM test kits to laboratories; training of laboratory staff; improving coordination between the EPI staff and the laboratory; assigning a unique identification number for each case; and installing computerized databases for surveillance and laboratories. It was noted that in 1999 the laboratory received 425 samples from clinicians for measles investigation and that these cases had not been reported to the Ministry of Public Health. It was found that 422 of the samples were measles IgM negative. Three were measles IgM positive but the children had been recently vaccinated.

10.5 Measles in Oman

Dr Suleiman Al Busaidy, National Poliovirus Laboratory, Oman

Oman has adopted the goal of measles elimination in the country by the year 2010. Prerequisites for achieving the goal are strong political commitment, high vaccination coverage, an elimination plan based on the local epidemiological situation, a strong disease surveillance system and strong laboratory support. Measles is a notifiable disease in Oman, regardless of the age of the suspected case. All health institutions in the public and private sector report and use standard clinical and laboratory case definitions. There is regular training and orientation of persons involved in the elimination programme. Surveillance indicators are monitored at the national level. Zero reporting for measles and rubella was established in 1999. There are 22 sentinel reporting sites among the Ministry of Health institutions and private clinics also report.

Measles vaccination started in Oman in 1981 with single antigen measles vaccine given at 9 months of age. Subsequently vitamin A supplementation was introduced in 1994 and measles and rubella vaccine (MR) was introduced in 1995 to be given at 15 months. In October 1997, MR vaccine was replaced by measles-mumps-rubella vaccine (MMR) given at 15 months. Measles vaccination coverage has been maintained at over 95% since 1990.

Large outbreaks of measles occurred in 1992 (1834 cases) and 1993 (3108 cases). Rapid transmission occurred in both outbreaks and mainly schoolchildren were affected. The

number of reported measles cases in Oman has declined since 1993 and the number of cases reported among Omanis was 2, 3, 1 and 6 for consecutive years between 1996 and 1999. Cases have also been detected among non-Oman residents numbering 10, 5, 0 and 6 between 1996 and 1999.

Since April 1996 there has been serological testing by IgM ELISA of samples from measles cases. Commercial kits are used. All samples are simultaneously screened for rubella IgM antibodies. A bank of positive sera is maintained. Virus isolation is done in B95a cells but so far no measles isolates have been obtained.

10.6 Measles in Kuwait

Dr Siham Al Mufti, Regional Reference Laboratory, Kuwait

Measles vaccination started in Kuwait in 1981 with single antigen measles vaccine given at 9 months of age. Subsequently MMR was introduced in 1983, but vaccination was optional and was given at 2 year of age. In 1998, MMR vaccination was made compulsory and it is given in 2 doses at the ages of 1 year and 4.5 years (i.e. at school entry).

Laboratory data were presented for the period 1994 to 2000, based on testing done at the Central Public Health Laboratory. The percentage of samples positive for measles declined from 59% of 94 samples tested in 1994 to 15% of 65 samples in 1999. The laboratory used complement fixation tests (CFTs) for measles serological investigations in the past. However more positive results are detected using IgM ELISA and there is a plan to discontinue testing by CFT.

There was a general decline in the number of reported measles cases in Kuwait between 1992 and 1999. However outbreaks of measles occurred in 1997 and 1998. In 1997 the majority of cases occurred in children less than 9 months of age and 16 of the 26 reported cases occurred in the region of Ahmadi. Ninety measles cases were reported in 1998 and the majority were detected in the regions of Hawalii (46 cases), Farwaniya (20 cases) and Al Jahra (18 cases).

11. CONCLUSIONS

The poliomyelitis eradication programme in the WHO Eastern Mediterranean Region is making greater use of virology investigations in the final classification of individual cases of acute flaccid paralysis. Genetic characterization of wild poliovirus isolates has become increasingly important in the investigation of transmission links among cases and in helping to identify wild virus reservoirs for targeting eradication efforts.

All poliovirus network laboratories in the Eastern Mediterranean Region were either fully or provisionally accredited by WHO in 1999. A substantial overall improvement in performance was noted in laboratories in Sudan and Iraq. It was particularly encouraging to note that several other laboratories improved performance and met standards for providing timely results. There was also a greater efficiency in systems for data management. All laboratories have functional computerized databases and access to mechanisms for weekly

electronic transfer of data to national and international partners involved in the regional poliomyelitis eradication programme.

In several countries there are lengthy delays between detection of polioviruses and referral of isolates to reference laboratories for characterization as wild or vaccine-like. Such delays have the potential to cause underestimation of the extent of wild poliovirus transmission and to impact adversely the planning of field surveillance and immunization activities to achieve poliomyelitis eradication. National authorities should place greater emphasis on identifying and finding solutions to problems related to sample referral.

No poliomyelitis cases have been detected for three or more years in 14 countries of the Region and rapid progress is being made in the remaining countries towards achieving the eradication goal. Laboratory support will continue to be required at least until global cessation of OPV immunization. In June 2000 the WHO Regional Director for the Eastern Mediterranean will inform ministries of health of the need to implement a regional plan to achieve laboratory containment of wild polioviruses and potentially infectious materials before certification of poliomyelitis eradication.

12. RECOMMENDATIONS

12.1 Role of network laboratories

1. WHO/EMRO should inform national authorities and other partners involved in poliomyelitis eradication that the role of the poliovirus network laboratories will continue beyond the interruption of wild poliovirus transmission and at least until global cessation of OPV immunization. Therefore national authorities should secure the necessary resources to support the poliovirus laboratory network at least until that time.

12.2 Intratypic differentiation of polioviruses

2. All poliovirus isolates detected in the WHO Eastern Mediterranean must be referred to reference laboratories for characterization as wild or vaccine-like as soon as possible and those from AFP cases must be referred within 14 days of detection. Poliovirus isolates from AFP cases should be shipped along with the original stool sample from which the isolate was obtained.

3. Regional reference laboratories should consider simultaneous testing (isolation and intratypic differentiation) of polio isolates and original stool samples from which they were obtained, whenever polioviruses are detected in non-endemic countries. Reference laboratories should consult with the regional laboratory coordinator to determine the priorities for simultaneous testing of isolates and original stools referred from endemic and recently endemic countries.

12.3 Laboratory quality assurance

4. WHO/HQ should be requested to develop guidelines on quality assurance for national and regional reference laboratories.

5. Reference laboratories must ensure that cells distributed to other network laboratories are tested for *Mycoplasma*. *Mycoplasma* testing of cell lines should be implemented in all regional reference laboratories by December 2001. In the interim, reference laboratories may access *Mycoplasma* testing services by referral of samples to other testing laboratories, as appropriate. Information about *Mycoplasma* negative status, inclusive of method used for testing, should be included in the documentation that accompanies cells when they are distributed.

6. Laboratory management should be included in the agenda of training activities for personnel in the poliovirus laboratory network, as appropriate.

12.4 Containment of wild polioviruses and potentially infectious materials

7. Initiation and implementation of national containment plans should be the responsibility of the ministries of health in collaboration with other relevant agencies.

8. National and regional laboratory staff should become familiar with WHO regional guidelines on containment and be prepared to serve in advocacy, advisory or supervisory roles in the containment process.

9. Poliovirus network laboratories should serve as models in the Region for implementing plans for containment of wild polioviruses and potentially infectious materials. These laboratories should implement phase 1 of the regional containment plan and submit an inventory of stored materials to the national certification committee and the WHO regional laboratory coordinator by December 2000.

10. Countries should consult with the WHO regional laboratory coordinator in making decisions about the disposition of any polioviruses and potentially infectious material.

11. Questions of enterovirus laboratories wishing to retain untyped or untypable strains should be resolved on a case-by-case basis in consultation with the WHO regional laboratory coordinator and with assistance from national or regional reference poliovirus laboratories, if needed.

12. Network laboratories should utilize the following guidelines for making containment decisions about poliovirus isolates and other potentially infectious materials:

- Original stool samples from all AFP cases should be retained at ≤ −20°C and stored in their original containers, for at least 12 months before discarding.
- Poliovirus isolates from AFP cases must be retained for at least 3 years.
- Poliovirus isolates identified as programmatically important may be retained at the request of regional or national authorities. Determination of programmatic importance will require consultation among EPI, laboratories and the regional laboratory coordinator.
- The only exception to the above guidelines for retaining original stools and poliovirus isolates will be for AFP cases whose samples have been referred to reference laboratories for further testing. In such situations, original stool samples, culture supernatants and stool suspensions may be discarded, but only after the reference or specialized laboratory has reported that materials were received in satisfactory condition and that all investigations were completed.

12.5 Measles laboratory support

13. Many of the laboratories involved in the poliomyelitis eradication initiative have also been designated to provide support for measles elimination programmes. National authorities, in consultation with WHO, should ensure that adequate human and financial resources are available to support the increased workload for measles laboratory diagnosis.

14. There should be a joint meeting of the directors of laboratories supporting poliomyelitis eradication and measles elimination programmes to foster better integration and planning of laboratory activities related to these programmes and to optimize the use of resources.

15. All countries that are implementing measles elimination programmes should designate laboratories to provide support to this activity by December 2000.

16. Measles virus isolates from the Region should be referred to specialized laboratories for genetic characterization to provide data for monitoring the progress of measles elimination activities.

Annex 1

PROGRAMME

Tuesday, 16 May 2000

08:30–09:00	Registration
09:00–09:20	Opening session Message from the Ministry of Health, Oman Message from the WHO Regional Director for the Eastern Mediterranean
09:20–09:30	Status regarding implementation of the recommendations of the third intercountry meeting of directors of poliovirus laboratories in the Eastern Mediterranean Region *Dr Esther de Gourville*
09:30–09:45	Progress towards global poliomyelitis eradication *Dr Raymond Sanders*
09:45–10:00	Progress towards poliomyelitis eradication in the WHO Eastern Mediterranean Region *Dr Faten Kamel*
10:00–10:15	Overview of wild poliovirus surveillance in the WHO Eastern Mediterranean Region *Dr Esther de Gourville*
10:15–10:30	Discussion

Laboratory reports on countries with wild poliovirus transmission

11:00–11:15	Report on Pakistan and Afghanistan *Dr Humayun Ashgar*
11:15–11:30	Report on Egypt *Ms Iman Nasr*
11:30–11:45	Report on the Islamic Republic of Iran *Dr Rakhshandeh Nategh*
11:45–12:00	Report on Iraq *Dr Walid Rida*
12:00–12:15	Report on Sudan/*Dr Isam El Khidir*
12:15–14:00	Discussion

Molecular epidemiology of wild polioviruses

14:00–14:30	Appropriateness of molecular sequencing in different epidemiological situations; the examples of Afghanistan, Egypt and Pakistan *Dr Mark Pallansch*
14:30–15:00	Molecular characterization of polioviruses from Iraq *Dr Harrie van der Avoort*
15:00–15:30	Discussion

Laboratory quality assurance-accreditation

16:00–16:15	Proficiency test performance in 1999 *Dr Harrie van der Avoort*

16:15–16:30	Current accreditation status of poliovirus laboratories in the WHO Eastern Mediterranean Region *Dr Esther de Gourville*
16:30–16:45	Overview of changes in the accreditation process and implications for network laboratories *Dr Raymond Sanders*
16:45–17:00	Discussion

Wednesday, 17 May 2000

Laboratory quality assurance-methodologies

08:30–09:00	Common problems and solutions in cell culture *Dr Glyn Stacey*
09:00–09:15	Validation results on characterized RD cell lines *Dr Mark Pallansch*
09:15–09:30	Description of the revised poliovirus laboratory manual *Dr Raymond Sanders*
09:30–09:40	Recommended techniques for intratypic differentiation of polioviruses in the global poliovirus laboratory network *Dr Raymond Sanders*
09:40–10:00	Discussion

Supplementary surveillance for wild polioviruses

10:00–10:20	Testing samples from non-polio AFP cases in Tunisia *Dr Hinda Triki*
10:20–11:00	Enterovirus surveillance in the Netherlands *Dr Harrie van der Avoort*
11:00–11:30	Challenges of implementing environmental surveillance *Dr Tapani Hovi*
11:30–11:45	Environmental surveillance in Morocco/*Dr Rajae Alouad*
11:45–12:00	Discussion

Laboratory management–equipment selection and performance

12:00–12:30	Equipment performance as an essential component of quality assurance in virology laboratories *Dr Glyn Stacey*
12:30–14:00	Discussion
14:00–14:15	Equipment concerns in Jordan/*Ms Karima Howaidy*
14:15–14:30	Equipment concerns in Saudi Arabia *Mr Moghram Al Amry*
14:30–14:45	Equipment concerns in the Syrian Arab Republic *Ms Halla Saba*
14:45–15:30	Discussion

Laboratory management–training of personnel

15:30–15:50	Demonstration of the updated Y2K compliant LABIFA software for data management in network laboratories *Dr Suleiman Al Busaidy*

15:50–16:00	Overview of training of personnel within poliovirus laboratories in the Region *Dr Esther de Gourville*
16:00–16:30	Discussion

Thursday, 18 May 2000

Containment of wild polioviruses

08:30–09:00	Progress towards implementation of the global containment plan for wild polioviruses *Dr Walter Dowdle*
09.00–09.15	Description of the regional plan for containment of wild polioviruses *Dr Esther de Gourville*
09:15–09:30	Experiences with implementing phase 1 of the containment plan in Oman *Dr Suleiman Al Busaidy*
09.30–09.45	Experiences with implementing phase 1 of the containment plan in Tunisia *Dr Hinda Triki*
09:45–10:30	Discussion

Measles laboratory activities

10:30–10:50	Regional progress towards measles elimination *Dr Faten Kamel*
10:50–11:15	Review of progress towards establishing a regional laboratory network to support measles elimination programmes *Dr Hinda Triki*
11:15–11:30	Measles laboratory activities in Tunisia *Dr Hinda Triki*
11:30–11:45	Measles laboratory activities in Oman *Dr Suleiman Al Busaidy*
11:45–12:00	Measles laboratory activities in Kuwait *Dr Siham Al Mufti*
12:00–13:00	Discussion
13:00	Conclusions and recommendations Closing session

Annex 2

LIST OF PARTICIPANTS

EGYPT
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Annex 3

RECOMMENDATIONS OF THE THIRD INTERCOUNTRY MEETING OF DIRECTORS OF POLIOVIRUS LABORATORIES IN THE WHO EASTERN MEDITERRANEAN REGION

1. Any polio isolations from non-endemic or recently endemic countries should be immediately notified to national authorities and the regional laboratory coordinator. Isolates in L20B cells that cannot be serotyped by recommended WHO techniques should also be notified, as they may also represent undetected polioviruses. Immediate notification is aimed at ensuring timely referral of isolates for intratypic differentiation, follow up, and resolving of administrative or other problems encountered with sample referral.

2. Poliovirus isolates and non typable L20B cell isolates should be referred to RRL within 14 days of identification. The isolates and the original stools from which they were obtained should be referred. Accompanying information must include: the EPID number that identifies the case, the province and district of origin, the serotyping results obtained in the referring laboratory, and information about whether isolates were stabilized through use of magnesium chloride.

3. Regional reference laboratories must immediately report results of ITD tests to the WHO Regional Office and the referring laboratory who should both inform the national EPI programme. Results of ITD tests should be provided in the standard line listing format.

4. Network laboratories, EPI staff and the regional laboratory coordinator should consult to identify and ensure referral of appropriate poliovirus isolates to specialized laboratories for genetic characterization. Isolates to be sequenced should include all wild polioviruses found in non-endemic countries as well as epidemiologically important isolates from endemic countries.

5. Virologists from poliomyelitis network laboratories should raise awareness of national authorities about the value of molecular epidemiology; how it complements information generated through conventional surveillance activities and how molecular epidemiology data can be effectively incorporated into programme planning, including supplemental immunization.

6. WHO/EMRO should recommend to WHO headquarters the production of training material on this subject for use in the Global Polio Laboratory Network.

7. To ensure the appropriate interpretation and use of molecular epidemiology information, specialized laboratories performing genetic sequencing should provide an interpretation along with the results of their findings.

8. Letters should be sent to the ministries of health of the Region informing them that weekly reporting of laboratory results to WHO will be implemented starting from the first week of June 1999.

9. The WHO Polio Laboratory Communications project should be fully implemented by December 1999 to ensure provision of e-mail linkages to all network laboratories, to facilitate rapid communication of laboratory results, and problem solving for matters related to technical performance and supplies.

10. The WHO Regional Office should plan and implement in-country training courses in EPI INFO and use of the Laboratory Information For Action (LABIFA) software to improve data quality and reporting frequency through increasing the number of network laboratory staff with computer skills.

11. National authorities should ensure the availability to network laboratories of computer support staff to assist in-country in the transition to computerized data management systems.

12. Network laboratories should ensure consultation with EPI staff before transmitting data to the Regional Office to reduce the discrepancies between reports from these two sources.

13. National authorities should ensure that meetings are held at least monthly between the EPI manager and the polio laboratory virologist to discuss issues of mutual concern.

14. The national polio laboratory virologist must be included in the national expert review committee to provide information and receive feedback about final case classification.

15. Virologists and EPI managers should both be included in regional poliomyelitis meetings to facilitate greater cooperation, exchange of technical information and joint partnership in all aspects of poliomyelitis eradication programmes.