

Annual reports

ANNUAL REPORT OF THE AUSTRALIAN NATIONAL ENTEROVIRUS REFERENCE LABORATORY 2012

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Abstract

In 2012 no cases of poliomyelitis were reported through clinical surveillance in Australia, and poliovirus was not detected through virological surveillance. Australia conducts surveillance for cases of acute flaccid paralysis (AFP) in children less than 15 years as the main mechanism to monitor its polio-free status in accordance with World Health Organization (WHO) recommendations. Cases of AFP in children are notified to the Australian Paediatric Surveillance Unit or the Paediatric Active Enhanced Disease Surveillance System. In 2012 Australia reported 1.2 non-polio AFP cases per 100,000 children, meeting the WHO performance criterion for a sensitive system for the fifth year in a row. However the faecal specimen collection rate from AFP cases was 29%, which was well below the WHO target of 80%. Virological surveillance for poliovirus consists of two components. Firstly, the Enterovirus Reference Laboratory Network of Australia (ERLNA) reports on the typing of enteroviruses detected in or isolated from clinical specimens. Secondly, environmental surveillance is conducted at sentinel sites. These surveillance systems are co-ordinated by the National Enterovirus Reference Laboratory (NERL).

Keywords: poliovirus, acute flaccid paralysis, surveillance, enterovirus, poliomyelitis, eradication, vaccination

Introduction

Australia, along with the World Health Organization (WHO) Western Pacific Region, was declared polio-free in 2000 and has established clinical and virological surveillance schemes to monitor its polio-free status. Clinical surveillance follows the WHO recommendation of investigating cases of acute flaccid paralysis (AFP) in children less than 15 years of age. AFP cases are ascertained either by clinicians notifying the Australian Paediatric Surveillance Unit (APSU) via a monthly report card or through the Paediatric Active Enhanced Disease Surveillance System (PAEDS). PAEDS involves ward based nurses reviewing hospital records and enrolling AFP patients with the consent of a parent or guardian at four sentinel tertiary paediatric hospitals.^{1,2} The WHO recommends that two faecal specimens be collected for virological investigation at least 24

hours apart and within 14 days of the onset of paralysis from cases of AFP in order to exclude poliovirus as the causative agent. It is a requirement of the WHO polio eradication program that specimens are tested in a WHO accredited laboratory, which for Australia is the National Enterovirus Reference Laboratory (NERL), formerly the National Polio Reference Laboratory (NPRL) at the Victorian Infectious Diseases Reference Laboratory (VIDRL). The clinical and laboratory data from AFP cases in children is reviewed by the Polio Expert Panel (PEP) and reported to the WHO as evidence of Australia's continued polio-free status.

Enterovirus and environmental surveillance programs were established as virological surveillance for poliovirus to complement the clinical surveillance program focussed on AFP cases in children. Enteroviruses other than poliovirus have been associated with AFP, and poliovirus infection may manifest clinically without paralysis. The Enterovirus Reference Laboratory Network of Australia (ERLNA) was established in 2009. Public diagnostic virology laboratories report their enterovirus typing results from clinical specimens to exclude poliovirus and establish the epidemiology of non-polio enteroviruses in Australia. WHO supports environmental surveillance as a sensitive means of detecting poliovirus through the testing of sewage samples. In December 2012, Egypt reported the detection of wild poliovirus type 1 in 2 sewage samples collected in Cairo.³ Genetic sequencing identified Pakistan as the source of the viruses.

The certification of India as being polio-free in January 2012 was a significant achievement for the global polio eradication program, reducing the number of endemic countries to three; Afghanistan, Nigeria and Pakistan.⁴ Furthermore, the reporting of 223 polio cases globally in 2012 represented the lowest number since the eradication program started in 1988.⁵ Nevertheless, it is important to maintain high polio vaccine coverage and sensitive surveillance systems for AFP cases in children until global eradication is achieved. As an example, China along with other countries of the Western Pacific Region, was declared polio-free in 2000. However, an outbreak in Xinjiang province in China due to a wild poliovirus type 1 importation from Pakistan caused 21 cases of polio before the country was declared polio-free once again in October 2012.⁴ A weekly situ-

ation report of polio cases worldwide is available at the WHO website <http://www.polioeradication.org/Dataandmonitoring/Poliothisweek.aspx>

This report summarises the polio surveillance program in Australia for 2012 encompassing clinical surveillance for AFP cases in children and virological surveillance for poliovirus.

Methods

AFP Surveillance

Paediatricians reviewing a patient less than 15 years of age presenting with AFP, or clinicians reviewing a patient of any age suspected of poliomyelitis, are requested to notify the NERL (telephone 03-9342 2607, email polio@mh.org.au). Paediatricians also notify the AFP case to the APSU (<http://www.apsu.org.au/>) via a monthly report card. Upon receipt of the notification, the AFP National Surveillance Co-ordinator based at VIDRL forwards a clinical questionnaire for the clinician to complete. Alternatively, AFP cases are ascertained by PAEDS nursing staff from medical records and are enrolled in the surveillance program with parental or guardian consent.

WHO classifies specimens as being adequate for virological investigation when two faecal specimens are collected more than 24 hours apart (due to intermittent virus shedding), and the specimens are collected within 14 days of the onset of paralysis (while the virus titre remains high). The faecal specimens are tested free of charge by the NERL.

The PEP, convened by the Department of Health (DoH), reviews the clinical and laboratory data for all notified cases of AFP, irrespective of whether they are an eligible or ineligible case. An eligible case is an Australian child under 15 years of age with AFP (including Guillain-Barré syndrome and transverse myelitis) or an Australian of any age with suspected polio. Ineligible cases include patients aged 15 years or older, overseas residents and cases notified in error or later determined not to be AFP.

The PEP classifies cases of AFP as:

- Poliomyelitis due to wild poliovirus, vaccine-derived poliovirus (VDPV) or vaccine associated paralytic poliomyelitis (VAPP);
- Polio compatible if there is insufficient evidence to exclude poliomyelitis;
- Non-polio AFP; or
- Non-AFP.

A follow-up questionnaire is sent to notifying clinicians if the PEP requires more information regard-

ing the AFP case before a final classification can be made. After each PEP meeting the Australian AFP case classifications are forwarded to WHO for inclusion in the global AFP surveillance data published in the Weekly Epidemiological Record (available at <http://www.who.int/wer/en/>). Ineligible cases are not reported to WHO.

The WHO AFP surveillance performance indicator for a polio non-endemic country is one case of non-polio AFP per 100,000 children aged less than 15 years. For Australia in 2012, this equated to 43 cases per year, based on the Australian Bureau of Statistics data released in December 2011. An AFP surveillance scheme that satisfies the surveillance performance indicator is deemed sufficiently sensitive to detect a wild poliovirus importation in children of that country. The WHO surveillance performance indicator for laboratory testing is that at least 80% of notified AFP cases have adequate faecal specimens collected and tested in a WHO accredited laboratory.

At the end of each calendar year a number of AFP notifications remain pending where insufficient clinical and laboratory data were made available to the PEP. The PEP classifies the remaining AFP notifications as “polio compatible-zero evidence” if a final review reveals no evidence of clustering amongst the cases.

Virus Culture

Upon receipt at the NERL, faecal specimens are treated with Minimum Essential Medium containing Hank's salts, chloroform (9.1% v/v) and foetal bovine serum (2%). The suspension is clarified and the supernatant inoculated onto a series of mammalian cell lines. Two WHO recommended cell lines are used for the isolation of poliovirus, L20B (a transgenic mouse epithelial cell line expressing the human poliovirus receptor, CD155) and RD-A (human rhabdomyosarcoma).^{6,7} Diagnostic laboratories in Australia are encouraged to refer enteroviruses of unknown serotype to the NERL for further characterisation as poliovirus infection can lead to clinical presentations without paralysis such as aseptic meningitis.

Two WHO real time reverse transcription polymerase chain reaction (RT-PCR) tests are used to determine whether a poliovirus is a wild strain, oral poliomyelitis vaccine (OPV) strain (Sabin-like) or a vaccine-derived poliovirus (VDPV), in a process known as intratypic differentiation.⁸ The NERL sequences the complete poliovirus viral protein 1 (VP1) genomic region, which contains a major neutralizing antibody binding site. The VP1 genomic sequence provides valuable biological information, including the number of mutations within a signifi-

cant region of the OPV virus strain and it enables phylogenetic analysis of wild poliovirus to rapidly determine the likely source of the virus, as utilised in the 2007 wild poliovirus importation.⁹

Enterovirus Surveillance

The ERLNA was established primarily as a means of detecting imported poliovirus amongst untyped enteroviruses from clinical specimens. The network consists of 10 public sector diagnostic virology laboratories in the Australian Capital Territory (Canberra Hospital), New South Wales (Royal Prince Alfred Hospital), Queensland (Queensland Health and Scientific Services), South Australia (Flinders Medical Centre, Institute of Medical and Veterinary Science), Tasmania (Royal Hobart Hospital), Victoria (Royal Children's Hospital, VIDRL) and Western Australia (Queen Elizabeth II Medical Centre, Princess Margaret Hospital for Children).

The NERL encourages members of the ERLNA to perform their own enterovirus typing. It has advised members of the ERLNA on enterovirus detection, supplied laboratory and computer analysis protocols and performed tests in parallel with other laboratories for quality assurance purposes. The NERL receives untyped enteroviruses from three laboratories for typing on a regular basis. The other laboratories perform their own enterovirus typing and report the results to the NERL for inclusion in the national enterovirus database.

The NERL screens clinical specimens for enteroviruses using a semi-nested RT-PCR directed to highly conserved sequence in the 5' non-translated region (NTR).¹⁰ Enterovirus typing is primarily performed by amplifying a fragment of the VP1 genomic region according to a published method,¹¹ but the complete nucleotide sequence of VP1 is required to type some enteroviruses. The enterovirus typing RT-PCR is directed to a region of sequence divergence that allows differentiation between enterovirus genomes. As a consequence, the enterovirus sequence based typing assay is not as sensitive as the pan-enterovirus detection assay. This can result in an enterovirus being detected by pan-enterovirus RT-PCR in a clinical specimen without subsequent identification by the VP1 enterovirus typing assay.

Environmental surveillance

The laboratory cell culture protocol implemented by the NERL for environmental surveillance is based on a two-phase separation procedure published by WHO and further advice was obtained from the Enterovirus Laboratory at the National Public Health Institute,¹² Finland, a Global Specialised Laboratory in the WHO Polio Laboratory Network. In brief, 800 mL of sewage is collected prior to any

biological or chemical treatment and referred to the NERL within 24 hours. At the laboratory 500 mL of the sample is centrifuged and the supernatant vigorously shaken at 4°C with dextran, polyethylene glycol and sodium chloride. The mixture is incubated overnight at 4°C in a separating funnel and the lower organic phase is collected the next day and used to re-suspend any pellet stored after the initial centrifugation. The final solution is clarified as for a faecal specimen and inoculated onto the L20B and RD-A cell lines and observed microscopically for cytopathic effect. The sewage extracts are tested in parallel by cell culture and a pan-enterovirus RT-PCR. The pan-enterovirus RT-PCR is a validated in-house test and is utilised to confirm the cell culture results as not all human enteroviruses can infect the RD-A cell line. All enterovirus isolates from cell culture and positive detections by RT-PCR were investigated to determine the virus type by nucleic acid sequencing.

Results

Classification of AFP cases

A total of 77 notifications of AFP in children less than 15 years of age were received in 2012 (Table 1). The PEP classified 51 cases as non-polio AFP with onset of paralysis in 2012. This equated to a non-polio AFP rate of 1.2 cases per 100,000 children less than 15 years of age, exceeding the WHO AFP surveillance performance criterion for a polio-free country of one case of non-polio AFP per 100,000 children (Table 2, Figure 1).

In 2012, one AFP case reviewed by the PEP had a differential diagnosis of Guillain-Barré syndrome and anterior horn cell disease due to an enteroviral infection. One faecal specimen, collected eight days after the onset of paralysis, was reported as no enterovirus isolated by cell culture by the NERL. The patient had not travelled overseas in the three months preceding the onset of symptoms. The PEP was not able to exclude polio based on the available clinical evidence and classified the case as "polio compatible" (Table 1). No further clinical information or laboratory specimens were received from one other AFP notification and the PEP classified the case as "polio compatible – zero evidence" to indicate the fact that it was a notification only with no further evidence to support a clinical diagnosis of polio.

Thirteen AFP cases were notified by more than one clinician and were regarded as duplicate notifications (Table 1). Eight AFP notifications did not meet the criteria for an eligible case. These involved either patients greater than 14 years of age, cases with symptom onset prior to 2012, or cases that were later reported as non-AFP. Three cases involving patients older than 14 years of age were all classified

by the PEP as non-polio AFP. However, they were not reported to the WHO as the global polio surveillance program focuses on AFP in children less than 15 years of age as the age group being at high risk of poliovirus infection.

Notification of AFP cases by state and territory

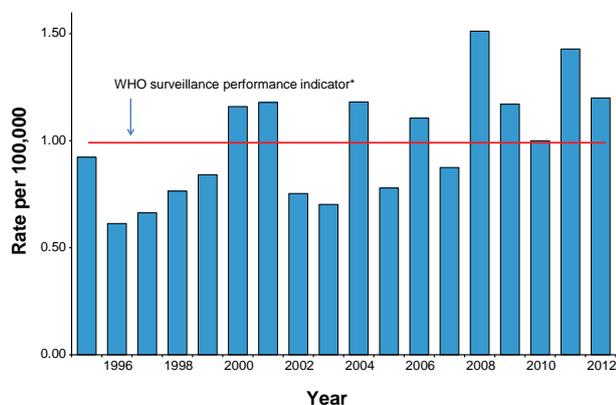
In 2012, eligible AFP cases were notified from all jurisdictions in Australia except the Australian Capital Territory and Tasmania (Table 1). The non-polio AFP rates for eligible cases per jurisdiction exceeded the WHO AFP surveillance performance indicator of one case per 100,000 children in New South Wales, Northern Territory, South Australia,

Victoria and Western Australia. Queensland was the only more populous state not to have achieved the WHO surveillance performance indicator.

Faecal collection from AFP cases

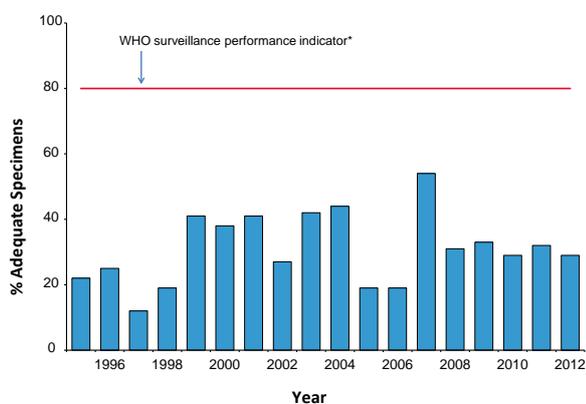
In 2012, a total of 64 faecal specimens from 37 of the 51 eligible cases were tested at the NERL (Table 3). No poliovirus was isolated from any of the specimens. The non-polio enteroviruses, coxsackievirus A7, coxsackievirus A16, coxsackievirus B5 and enterovirus 71 subgenogroup C2 were reported from four AFP cases in 2012. Diagnoses were transverse myelitis for the first two cases, Guillain-Barré syndrome for the third and acute disseminated encephalomyelitis for the last. Fifteen (29%) of the eligible cases had adequate specimens collected in 2012, while another 12 (24%) cases had only one specimen collected within the optimal period. This compares to the further WHO AFP surveillance criterion that 80% of the eligible AFP cases should have adequate specimens collected, a result that Australia has never achieved nationally (Figure 2). At the jurisdictional level, Queensland was the only state to reach the WHO target in 2012, with adequate specimens collected from all five cases classified (100%).

Figure 1: Non-polio AFP rate classified by the PEP, 1995 to 2012



* The WHO AFP surveillance performance indicator for a polio non-endemic country is one case per 100,000 children <15 years of age.

Figure 2: Percentage of AFP cases with adequate faecal specimens, 1995 to 2012



* The criterion for the WHO surveillance performance indicator is the collection of two faecal specimens more than 24 hours apart and within 14 days of the onset of paralysis from 80% of classified non-polio AFP cases.

Enterovirus and environmental surveillance

No poliovirus was detected by enterovirus or environmental surveillance in 2012. The ERLNA typed 277 non-polio enteroviruses with coxsackievirus A6 and echovirus 18 amongst the most frequent detections in Australia during the year (Table 4).

Four collections from each of the three sentinel sites (Armidale, Byron Bay and Newcastle) were tested by RT-PCR, and virus isolation. Twelve collections (four from each site) were tested by cell culture and RT-PCR. All 12 samples were positive by pan-enterovirus RT-PCR and non-polio enterovirus was isolated in cell culture from eight samples. Four samples positive by pan-enterovirus RT-PCR could not be typed due to low virus titres.

Regional reference laboratory activities

Several activities were performed as a Polio Regional Reference Laboratory in 2012. Specimens from AFP cases were referred from Brunei Darussalam (2 cases), Pacific Island countries (6 cases) and Papua New Guinea (9 cases). No poliovirus was isolated from any of the specimens but non-polio enteroviruses were reported from one case from the Pacific Islands and 5 cases from Papua New Guinea.

Six poliovirus type 2 and eight poliovirus type 3 isolates were referred from AFP cases in the Philippines for intratypic differentiation and all were character-

Table 1: Notification of AFP cases in Australia, 2012, by state or territory

State/ Territory	Estimated population aged <15 years	Expected number of AFP cases†	Total number of AFP cases	Ineligible notifications	Duplicate notifications	Polio compatible-zero evidence	Polio compatible	Pending	Eligible cases with final classification by PEP	Non-polio AFP rate per 100,000 children
ACT	67,397	0.5	0	0	0	0	0	0	0	0.0
NSW	1,358,279	14	21	1	2	0	1	1	16	1.1
NT	52,749	0.5	2	0	0	0	0	0	2	4.0
QLD	909,482	9	14	2	4	1	0	2	5	0.6
SA	293,392	3	5	0	0	0	0	0	5	1.7
TAS	97,694	1	1	1	0	0	0	0	0	0.0
VIC	1,027,417	10	28	4	6	0	0	0	18	1.8
WA	453,747	5	6	0	1	0	0	0	5	1.0
Australia	4,260,157	43	77	8	13	1	1	3	51	1.2

* Australian Bureau of Statistics, estimated population at 30 June 2011. Available at <http://www.abs.gov.au/>

† Based on a non-polio AFP rate of 1 case per 100,000 children less than 15 years of age

Table 2: Surveillance for AFP cases in children less than 15 years, Australia, 2012, compared with the WHO performance indicators

WHO surveillance performance indicator for AFP cases in children <15 years	Performance of Australia's AFP surveillance	
	Number of cases/specimens 2012	Comparison with WHO indicator 2012
AFP cases		
≥1.0 non-polio AFP case / 100,000 children (43 cases for Australia in 2012).	51 cases classified as non-polio AFP	1.2 (51 / 43) non-polio AFP cases / 100,000 children <15 years
Adequate specimen collection		
≥80% of classified AFP cases with adequate specimens* (41 cases for Australia in 2012).	15 AFP cases with adequate specimens collected	29% (15 / 51) classified non-polio AFP cases with adequate specimens

* Adequate specimen collection is defined as 2 faecal specimens collected at least 24 hours apart and within 14 days of onset of paralysis

ised as being Sabin-like. Seven poliovirus type 1 and two poliovirus type 2 isolates were characterised as Sabin-like from sources other than AFP.

Quality Assurance Programs

In 2012, the NERL passed the annual WHO quality assurance panels for poliovirus isolation by cell culture and poliovirus RT-PCR for intratypic differentiation and vaccine derived poliovirus. The WHO distributed the first official poliovirus

sequencing proficiency panel and the laboratory scored full marks for sequencing RNA templates consisting of wild, Sabin and Sabin prototype mixtures of poliovirus. The laboratory also participated in the Royal College of Pathologists of Australasia quality assurance panel for enterovirus detection by RT-PCR.

Table 3: Specimens referred to the NERL Australia, 2012

Result	Specimens from AFP cases in patients < 15 years of age	Specimens from AFP cases in patients ≥15 years of age	Specimens from sources other than AFP	TOTAL
Non-polio enterovirus	5	0	150	155
Rhinovirus	1	0	2	3
No enterovirus identified	58	4	35	97
Total	64	4	187	255

Table 4: Enterovirus test results from the NERL Australia, 1995 to 2012

Year	Poliovirus		Non-polio enterovirus	No enterovirus detected	EVID results referred [†]	Total samples reviewed
	Sabin-like	Non-Sabin-like*				
1995	190	0	200	13	0	403
1996	224	0	198	9	0	431
1997	124	0	76	0	0	200
1998	52	0	15	4	0	71
1999	60	1	9	9	0	79
2000	45	0	44	47	0	136
2001	46	5	33	75	0	159
2002	36	0	21	49	0	106
2003	9	0	15	47	0	71
2004	6	0	26	61	0	93
2005	18	0	10	39	0	67
2006	2	0	6	71	29	108
2007	0	2	32	115	107	256
2008	0	0	20	92	77	189
2009	1	0	63	78	113	255
2010	0	0	170	39	108	317
2011	0	0	174	61	205	440
2012	0	0	155	97	123	375

* Untyped enterovirus or uncharacterised poliovirus isolates were referred for further testing after completion of a laboratory inventory in 1999 and 2001; the six non-Sabin-like isolates were identified as wild type poliovirus prototype strains and destroyed. Wild poliovirus type 1 was imported from Pakistan in 2007. A Sabin-like poliovirus type 1 was identified from an unimmunised infant in 2009.

† Enterovirus Identification (EVID) results include retrospective data made available via the ERNLA.

Discussion

In 2012, Australia reached the WHO surveillance target of ≥ 1 non-polio AFP case per 100,000 children, for the fifth year in a row. The continued participation of clinicians and health care workers in notifying cases of AFP to the APSU and VIDRL along with the involvement of the ward based nurses in the PAEDS is essential in reaching this target, indicative of a sensitive surveillance system. Collection of adequate faecal specimens from AFP cases in Australia has never met the WHO surveillance target and represents a gap in clinical surveillance for imported cases of polio. The establishment of supplementary virological surveillance for poliovirus by the typing of enteroviruses and testing of environmental samples at sentinel sites provides an additional means of monitoring Australia's polio-free status.

Since the initial target for global polio eradication by the year 2000 set by the World Health Assembly (WHA) in 1988, a number of subsequent target years set for achieving polio eradication have not been met, the most recent being the end of 2012.¹³ However significant achievements have been attained since 1988, including the last reported case of wild poliovirus type 2 in 1999 and a reduction in the number of polio endemic countries worldwide from 125 to 3 by 2012. However, cases of wild poliovirus have reviewed around 1,200 annually between 2002 and 2010 (Figure 3).¹⁴ After Egypt was certified polio-free in 2006, it has proved difficult to eradicate the virus from the remaining areas of wild poliovirus transmission (Afghanistan, India, Nigeria and Pakistan).

A new strategy was initiated from 2005 with the introduction of monovalent oral polio vaccine for poliovirus type 1 and poliovirus type 3 followed by bivalent oral polio vaccine for poliovirus types 1 and 3 in 2009.¹⁵ The judicious use of trivalent, bivalent

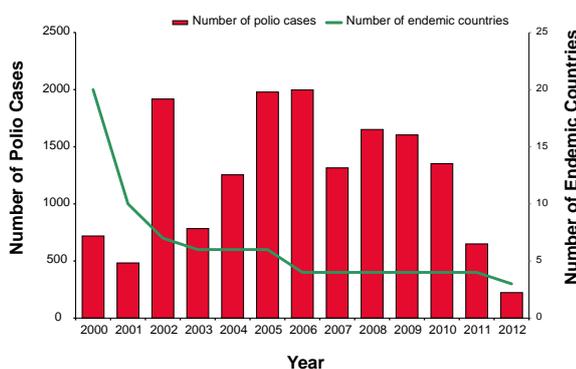
and monovalent oral polio vaccines reduced the number of polio cases in the polio endemic countries and those with re-established transmission to 217 and 6, respectively, in 2012.⁵ Changes to the WHO laboratory testing protocols were also introduced from 2006 to shorten the time taken to confirm cases of polio.¹⁶ The WHO Global Polio Laboratory Network introduced a new cell culture algorithm that halved the reporting time from 28 days to 14 days and implemented real time RT-PCR protocols that reduced the timeframe for poliovirus intratypic differentiation from 14 days to 7 days.

A further response in 2010 was the establishment of an Independent Monitoring Board (IMB) by the WHA to monitor and guide the progress of the Global Polio Eradication Initiative's 2010-2012 Strategic Plan.¹⁷ The IMB was convened quarterly to review developments in the polio program and provided independent advice regarding the requirements for the plan to succeed. The 2010 to 2012 strategic plan aimed to stop wild poliovirus transmission in two of the four endemic countries by the end of 2011, with only one country, India, achieving that goal.⁴ Considering that India reported 741 cases of polio as recently as 2009, representing 46% of the cases worldwide, its certification as polio-free in January 2012 was a significant milestone for the eradication program. Despite this, the January 2012 IMB report concluded that global polio eradication would not be achieved if it continued on its current path.¹⁷ One of the board's conclusions was to place a greater emphasis on people management, including rating the importance of having well-trained vaccinators who are valued and inspired as the most important group in the programme. The cessation of polio vaccination in response to the deliberate killing of polio vaccinators in Pakistan in late 2012 reinforced the board's opinion of their key role.¹³

In January 2012, in response to a recommendation by the IMB, the WHO Executive Board called "the completion of poliovirus eradication a programmatic emergency for global public health". This was adopted as a resolution by the WHA the following May.¹⁸ By the end of 2012, 223 cases of polio were reported for the year, the lowest annual total ever (Figure 3). Transmission of wild poliovirus was restricted to four countries, the lowest since the program began; the three endemic countries of Afghanistan, Nigeria and Pakistan, and Chad with re-established transmission.⁵ Furthermore, wild poliovirus type 3 was last reported in Afghanistan and Pakistan in April 2012, potentially leaving Nigeria as the last country to be endemic for this serotype.

The Polio Eradication Initiative appears to be at a critical juncture. A concerted international effort is required to support the final stages of wild

Figure 3: Cases of wild polio virus infection in endemic countries, 2000 to 2012, by year



Data from: <http://www.polioeradication.org/Dataandmonitoring/Poliothisweek/Wildpolioviruslist.aspx>, accessed 27 March 2013.

poliovirus eradication to avoid a repeat of 2001. This was the last time that relatively few cases were reported, and was followed in subsequent years by a rapid rise in the number of cases in the endemic countries and frequent occurrences of re-established transmission in others. To restrict the international spread of wild poliovirus, the IMB recommended in its seventh report that by May 2013 the International Health Regulations Expert Review Committee issue a standing recommendation that travellers from the remaining endemic countries receive pre-travel vaccination or a check of their vaccination status until polio transmission in the country ceases.¹⁷

Until certification of global wild poliovirus eradication, Australia remains at risk of an importation as occurred from Pakistan in 2007.⁹ The continued performance of the clinical and virological surveillance systems for poliovirus to a high international standard is essential to monitor Australia's polio-free status in order to detect and rapidly respond to any future polio importation event.

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References

1. Study Protocol, Acute Flaccid Paralysis. Australian Paediatric Surveillance Unit [Internet]. Accessed on 27 March 2013. Available from: <http://www.apsu.org.au/assets/current-studies/AFP-Study-Protocol-APSU-Final-110810.pdf>.
2. Paediatric Active Enhanced Disease Surveillance [Internet]. Accessed on 27 March 2013. Available from: <http://www.apsu.org.au/surveillance-systems/paeds/>.

3. Outbreak news – Poliovirus isolation, Egypt. *Weekly Epidemiological Record*. 2013;88:74-75.
4. World Health Organization. Progress towards global interruption of wild poliovirus transmission, January 2011–March 2012. *Weekly Epidemiological Record*. 2012;87:189-200.
5. Global Polio Eradication Initiative [Internet]. Wild poliovirus 2008-2013. Accessed on 27 March 2013. Available from: http://www.polioeradication.org/Portals/0/Document/Data&Monitoring/Wild_poliovirus_list_2008_2013_26Mar.pdf
6. Wood DJ, Hull B. L20B Cells Simplify Culture of Polioviruses from Clinical Samples. *J Med Virol* 1999;58:188-192.
7. World Health Organization. *Polio Laboratory Manual, 4th edition*, WHO/IVB/04.10. Geneva: Department of Immunization, Vaccines and Biologicals; 2004.
8. Kilpatrick DR, Yang CF, Ching K, Vincent A, Iber J, Campagnoli R, Mandelbaum M, De L, Yang SJ, Nix A. Rapid group-, serotype-, and vaccine strain-specific identification of poliovirus isolates by real-time reverse transcription PCR using degenerate primers and probes containing deoxyinosine residues. *J Clin Microbiol* 2009;47:1939-1941.
9. Stewardson AJ, Roberts JA, Beckett CL, Prime HT, Loh PS, Thorley BR, Daffy JR. An Imported Case of Poliomyelitis in Melbourne, Australia. *Emerging Infect. Dis.* 2009;15:63-65.
10. Roberts JA, Thorley BR. Enterovirus. In: Schuller M, Sloots TP, James GS, Halliday CL, Carter IWJ, editors. *PCR for Clinical Microbiology: An Australian and International Perspective*. 1st edn. Dordrecht: Springer; 2010. p. 229-233.
11. Nix WA, Oberste MS, Pallansch MA. Sensitive, seminested PCR amplification of VP1 sequences for direct identification of all enterovirus serotypes from original clinical specimens. *J Clin Microbiol* 2006;44:2698-2704.
12. World Health Organization. Guidelines for environmental surveillance of poliovirus circulation. Geneva: Department of Vaccines and Biologicals WHO/V&B/03.03; 2003.
13. Global polio eradication: not there yet [editorial]. *Lancet* 2013;381:1.
14. Global Polio Eradication Initiative [Internet]. Wild poliovirus list. Accessed on 27 March 2013. Available from: <http://www.polioeradication.org/Dataandmonitoring/Poliothisweek/Wildpolioviruslist.aspx>.
15. Crawford NW, Buttery JP. Poliomyelitis eradication: another step forward. *The Lancet* 2010;376:1624-1625.
16. World Health Organization. Informal consultation of the Global Polio Laboratory Network-June 2008. *Weekly Epidemiological Record* 2008;83:261-268.
17. Independent Monitoring Board [Internet]. IMB meeting reports. Accessed on 27 March 2013. Available from: <http://www.polioeradication.org/Aboutus/Governance/IndependentMonitoringBoard/Reports.aspx>
18. Global Polio Eradication Initiative [Internet]. Agenda item 13.10. Poliomyelitis: intensification of the global eradication initiative. WHA65.5. May 2012. Accessed 27 March 2013. Available from <http://www.polioeradication.org/ResourceLibrary/Declarationsandresolutions.asp>