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Australian National Enterovirus Reference Laboratory annual report, 2016

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Abstract

Australia monitors its polio-free status by conducting surveillance for cases of acute flaccid paralysis (AFP) in children less than 15 years of age, as recommended by the World Health Organization (WHO). Cases of AFP in children are notified to the Australian Paediatric Surveillance Unit or the Paediatric Active Enhanced Disease Surveillance System and faecal specimens are referred for virological investigation to the National Enterovirus Reference Laboratory. In 2016, no cases of poliomyelitis were reported from clinical surveillance and Australia reported 1.38 non-polio AFP cases per 100,000 children, meeting the WHO performance criterion for a sensitive surveillance system. Several non-polio enteroviruses, coxsackievirus A6, enterovirus A71, enterovirus A74 and enterovirus D68, were identified from clinical specimens collected from AFP cases. The global withdrawal of Sabin poliovirus type 2 from oral polio vaccine occurred in April 2016. This event represents the start of the polio endgame with an increased focus on the laboratory containment of all remaining wild and vaccine strains of poliovirus type 2. The National Enterovirus Reference Laboratory was designated as a polio essential facility as part of this process. In 2016, 37 cases of wild polio were reported with three countries remaining endemic: Afghanistan, Nigeria and Pakistan. Nigeria was declared polio-free in 2015, after 12 months without detection of wild poliovirus, but was reinstated as an endemic country after the reporting of four cases in August 2016. This is a salient reminder of the need to maintain sensitive surveillance for poliovirus until global eradication is certified.

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

Introduction

Australia has established clinical and virological surveillance schemes to monitor its polio-free status. The clinical surveillance follows the World Health Organization (WHO) recommendation of investigating cases of acute flaccid paralysis (AFP) in children less than 15 years of age as an age group at high risk of poliovirus infection. 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) at five sentinel tertiary paediatric hospitals.^{1,2,3} 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 to exclude poliovirus as the causative agent. It is a requirement of the WHO polio eradication program that the specimens are tested in a WHO-accredited laboratory, which for Australia is the National Enterovirus Reference Laboratory (NERL) 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 clini-

cal 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 such as meningitis. The Enterovirus Reference Laboratory Network of Australia (ERLNA) involves public diagnostic virology laboratories reporting enterovirus typing results from clinical specimens to exclude poliovirus involvement and establish the epidemiology of non-polio enteroviruses (NPEVs) in Australia. Most poliovirus infections are asymptomatic with the virus shed for weeks in the faeces of infected persons. WHO supports the testing of environmental or raw sewage samples as a means of detecting the presence of wild poliovirus in polio-free countries.

The number of wild polio cases worldwide decreased from 74 in 2015 to 37 in 2016.⁴ Afghanistan and Pakistan accounted for most of the cases, with 13 and 20 reported respectively. Nigeria was declared polio-free in September 2015, after more than 12 months with no detection of wild poliovirus, however four cases were reported in August 2016 in security-compromised areas.⁵ Only wild poliovirus serotype 1 was detected in 2016, with the last report of wild poliovirus type 3 in Nigeria in November 2012.⁶ The global eradication of wild poliovirus type 2 was certified in September 2015, with the last detection reported in India in 1999.⁷ This achievement led to the globally synchronised withdrawal of Sabin 2 poliovirus from OPV along with laboratory containment of this serotype from 2016, which involves restricted access at a limited number of facilities worldwide.⁸ The three poliovirus serotypes will remain in the inactivated polio vaccine and all countries will incorporate at least one dose of inactivated polio vaccine in the routine immunisation schedule to maintain immunity to poliovirus type 2 with the switch from trivalent OPV to bivalent OPV in April 2016.⁹

Vaccine-derived poliovirus can cause outbreaks of paralytic polio and in 2016, cases caused by circulating strains were reported in Laos, Nigeria and Pakistan.¹⁰ WHO have declared

the international spread of wild poliovirus and circulating vaccine-derived poliovirus to be a Public Health Emergency of International Concern since May 2014. The situation has been assessed every three months since then and the declaration has remained in place with countries known to be exporting wild poliovirus and circulating vaccine-derived poliovirus required to ensure all residents and long-term visitors are vaccinated between four weeks and 12 months prior to international travel.¹¹

This report summarises the polio surveillance program in Australia for 2016, encompassing clinical surveillance for AFP cases in children and virological surveillance for poliovirus.

Methods

Acute flaccid paralysis surveillance

Paediatricians reviewing a patient less than 15 years of age presenting with AFP, or clinicians reviewing a patient of any age with suspected poliomyelitis, are requested to notify the NERL.ⁱ Poliovirus infection, including suspected poliomyelitis, is notifiable under the Nationally Notifiable Disease Surveillance System.¹² Paediatricians notify AFP cases to the APSUⁱⁱ via a monthly report card. Upon receipt of the notification, the AFP National Surveillance Co-ordinator based within the NERL 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.

According to the WHO surveillance criterion, two faecal specimens must be collected more than 24 hours apart due to intermittent virus shedding, and within 14 days of the onset of paralysis, while the virus titre remains high, to be classified as adequate. The faecal specimens are tested free of charge by the NERL.

i telephone 03-9342 9607, email enterovirus@mh.org.au

ii <http://www.apsu.org.au/>

The PEP, a subcommittee of the Communicable Disease Network of Australia, 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 less than 15 years of age with AFP (including Guillain-Barre syndrome and transverse myelitis) or an Australian of any age with suspected polio.

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.

The clinician is contacted if the PEP requires more information regarding 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*.ⁱⁱⁱ Ineligible cases are not reported to WHO.

The WHO AFP surveillance performance indicator for a polio non-endemic country is 1 case of non-polio AFP per 100,000 children aged less than 15 years.¹³ For Australia in 2016, this equated to 45 cases, based on the Australian Bureau of Statistics data released in December 2015. An AFP surveillance scheme that satisfies the WHO 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.

iii available at <http://www.who.int/wer/en/>

Virus culture

Upon receipt at the NERL, faecal specimens are treated with minimum essential medium containing Earle's salts, chloroform (9.1% v/v) and phosphate buffered saline. The suspension is clarified and the supernatant inoculated onto the two mammalian cell lines recommended by WHO for the isolation of poliovirus: L20B (a transgenic mouse epithelial cell line expressing the human poliovirus receptor, CD155) and RD-A (human rhabdomyosarcoma).^{14,15}

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 (ITD).¹⁶ The NERL sequences the complete poliovirus viral protein 1 (VP1) genomic region, which contains a major neutralising antibody binding site. The VP1 genomic sequence provides valuable biological information, including the number of mutations within a significant 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.¹⁷

Enterovirus surveillance

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

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 four 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 enterovirus using a semi-nested RT-PCR directed to highly conserved sequence in the 5' non-translated region (NTR).¹⁸ Enterovirus typing is primarily performed by amplifying a fragment of the VP1 genomic region according to a published method,¹⁹ 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.

Results

Classification of AFP cases

A total of 96 notifications of AFP cases involving children less than 15 years of age were received in 2016 (Table 1). The PEP classified 62 cases as non-polio AFP, a rate of 1.38 cases per 100,000 children less than 15 years of age, which exceeds 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). Transverse myelitis and Guillain-Barré syndrome were the most common cause of non-polio AFP in 2016, with the PEP classifying 16 cases each with these two conditions.

One of the AFP cases from Queensland was classified by the PEP as polio-compatible. While the final diagnosis was myeloradiculitis, the patient's condition was suggestive of poliomyelitis with anterior horn cell inflammation of the spinal cord and no stool specimens were collected to confirm or exclude poliovirus infection. The case was included in the non-polio AFP data in Table 1 to be consistent with the reporting system used by WHO for Australia.

Twenty-eight cases were notified by more than one source, whether by two or more clinicians or a clinician and the PAEDS system. Six notifications were deemed to be ineligible due to the patient's age being greater than 14 years or the clinical presentation was subsequently determined not to be AFP.

Notification of AFP cases by state and territory

In 2016, AFP cases were notified from all jurisdictions in Australia except the Australian Capital Territory (Table 1). This result may not be surprising, since the Australian Capital Territory is expected to report one case every one to two years based on the population less than 15 years of age, however, no AFP cases have been notified from this jurisdiction since 2009. The non-polio AFP rates for eligible cases by jurisdiction exceeded the WHO AFP surveillance performance indicator of one case per 100,000 children in all other states and territory except Western Australia with a non-polio AFP rate of 0.80 per 100,000 children less than 15 years of age.

Faecal collection from AFP cases

A total of 90 faecal specimens from 51 of the 62 eligible cases were tested at the NERL in 2016. Twenty-eight AFP cases met the WHO criterion for specimen testing with two faecal specimens collected within 14 days of the onset of paralysis, representing 45% of the cases classified as non-polio AFP compared to the WHO criterion of 80% of cases (Figure 2, Table 2). The proportion of cases with at least one specimen collected

Table 1: Notification of acute flaccid paralysis cases, 2016 by state or territory.

State or territory	Estimated population aged <15 years ^a	Expected number of AFP cases in 2016	Total number of notifications	Ineligible notifications	Duplicate notifications	Eligible cases with final classification by PEP ^b	Non-polio AFP rate per 100,000 children
ACT	73,674	1.0	0	0	0	0	0.00
NSW	1,427,380	14.0	19	2	1	16	1.14
NT	53,990	0.5	1	0	0	1	2.00
Qld	944,386	9.5	31	1	16	14	1.47
SA	298,953	3.0	8	0	1	7	2.33
Tas	94,182	1.0	2	0	0	2	2.00
Vic	1,085,207	11.0	31	3	10	18	1.64
WA	497,775	5.0	4	0	0	4	0.80
Australia	4,475,547	45.0	96	6	28	62	1.38

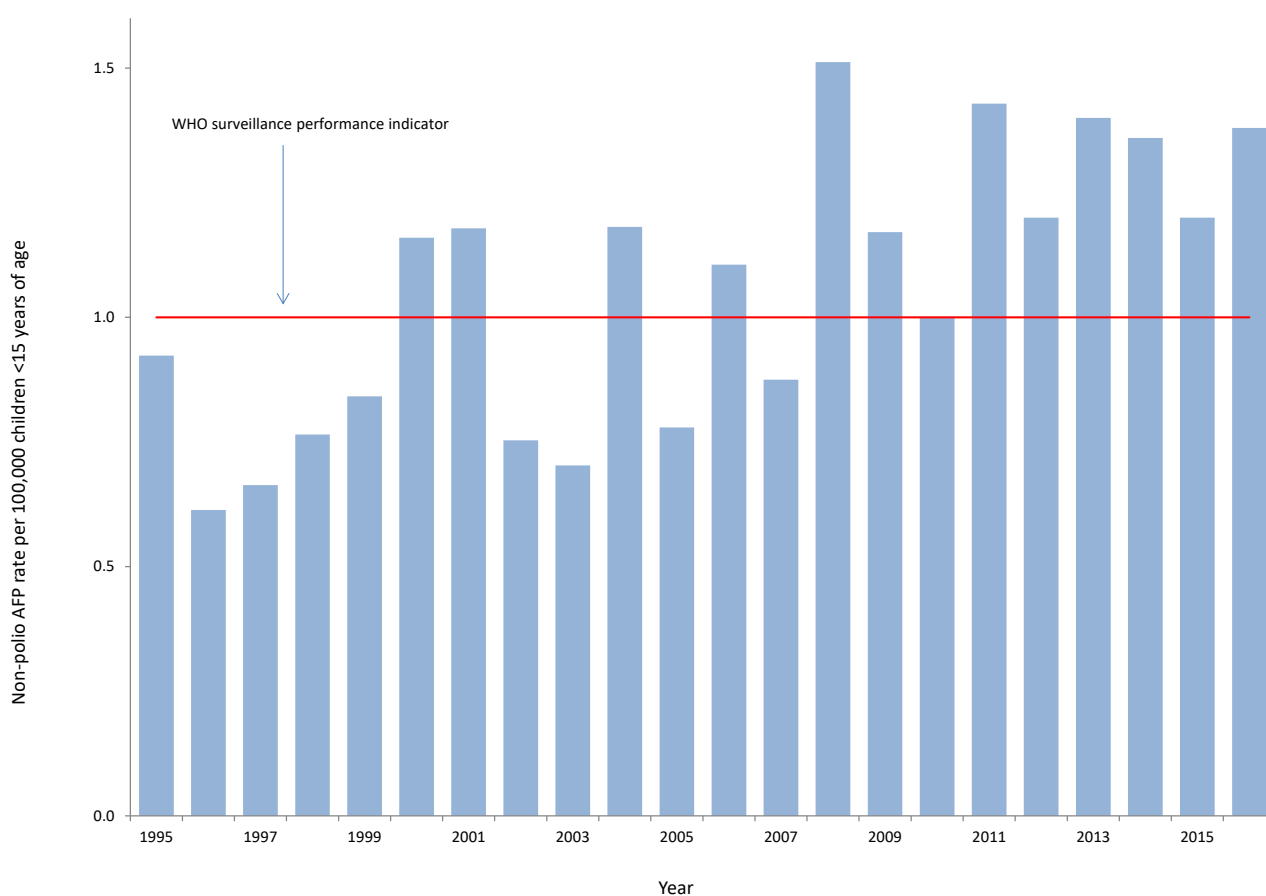
a Australian Bureau of Statistics, estimated population at 30 June 2015. Available at www.abs.gov.au.

b Includes one case classified as polio-compatible in Queensland.

Table 2: Australia’s surveillance for cases of acute flaccid paralysis, 2016, compared with the main World Health Organization performance indicators

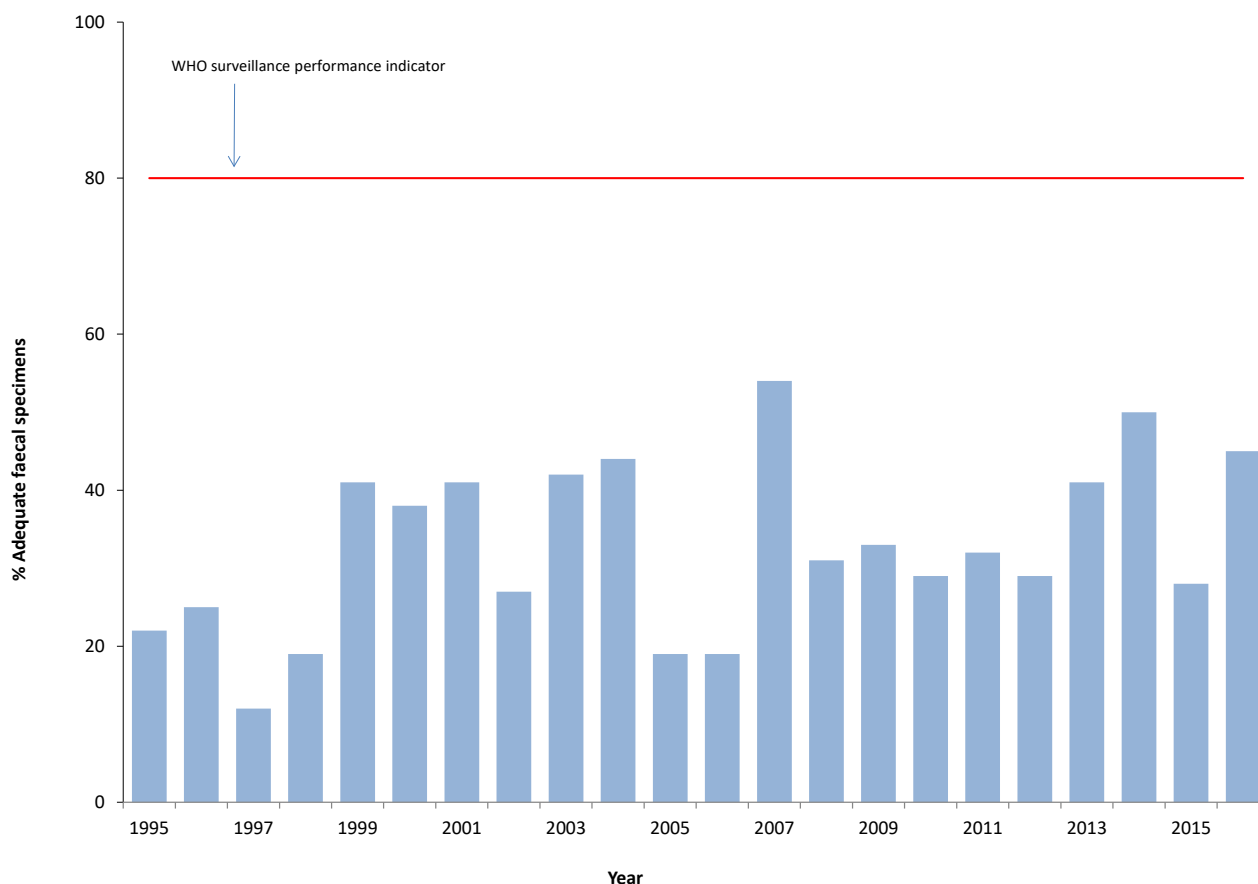
WHO surveillance performance indicator for AFP cases in children <15 years	Performance of Australia’s AFP surveillance	
≥1.0 non-polio AFP case / 100,000 children (44 cases for Australia in 2016)	62 cases classified as non-polio AFP	1.38 (62 / 45) non-polio AFP cases / 100,000 children <15 years
≥80% of classified AFP cases with adequate specimens (2 faecal specimens collected at least 24 hours apart and within 14 days of onset of paralysis)	28 AFP cases with adequate specimens collected	45% (28 / 62) classified non-polio AFP cases with adequate specimens

Figure. 1. Non-polio acute flaccid paralysis rate, Australia 1995 to 2016^a



^a The WHO AFP surveillance performance indicator for a polio non-endemic country is one case per 100,000 children <15 years of age, which is indicated by the red line.

Figure 2. Adequate faecal specimen rate, Australia 1995 to 2016^a



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

within 14 days of the onset of paralysis was 69%, while 73% of cases had a specimen collected any time after the onset of paralysis. No poliovirus was detected in any of the specimens. The following non-polio enteroviruses were identified from stool specimens collected from non-polio AFP cases with the jurisdiction indicated in brackets: coxsackievirus A6 (NSW), enterovirus A71 (two cases in Victoria with the onset of paralysis seven months apart), enterovirus A74 (South Australia) and enterovirus D68 (South Australia).

Enterovirus surveillance

A total of 235 NPEVs were typed by the NERL and an additional 227 by members of the Enterovirus Reference Laboratory Network of Australia from clinical specimens (Tables 3 and 4). No polioviruses were reported amongst

the enterovirus typings. The most common genotypes of NPEV identified by the laboratory network, in order of decreasing frequency, were echovirus 18, coxsackievirus A6, echovirus 6 and coxsackievirus B4.

Polio regional reference laboratory activities

As part of its role as a Polio Regional Reference Laboratory, in 2016, the NERL received specimens from AFP cases referred from Brunei Darussalam (1 case), Pacific Island countries (15 cases) and Papua New Guinea (27 cases). Sabin-like poliovirus type 3 was isolated from one AFP case from Papua New Guinea, which was likely to have been associated with a recent vaccination event. NPEVs were reported from seven AFP cases from the Pacific Islands and 11 cases from Papua New Guinea.

Table 3: Specimens referred to the NERL Australia, 2016

Result	Specimens from AFP cases involving children < 15 years of age	Specimens from AFP cases involving patients ≥15 years of age	Enterovirus Surveillance	Total
Non-polio enterovirus	7	0	235	242
No enterovirus identified	83	1	59	143
Total	90	1	294	385

Quality assurance programs

In 2016, the NERL was accredited as a WHO Polio Regional Reference Laboratory through participation in the annual WHO quality assurance panels for poliovirus isolation, intratypic differentiation and sequencing. The laboratory also successfully participated in the Royal College of Pathologists of Australasia quality assurance panel for enterovirus detection by RT-PCR.

Discussion

Australia has met the WHO non-polio AFP surveillance target for the ninth year in a row, reporting 1.38 cases per 100,000 children less than 15 years of age. The notification of AFP cases via the APSU monthly report card and the PAEDS system has routinely met the international standard that assesses whether an imported case of polio in children less than 15 years of age would be detected, although gaps in AFP surveillance were noted at the sub-national level in the Australian Capital Territory and Western Australia, based on the WHO surveillance target.^{1,2,3,13} An investigation of AFP case reporting at the SA4 level, the largest substate region, from 2001 to 2015, was initiated by the National Certification Commission for Poliomyelitis Eradication in 2016 (*in press*). The study found 9% of regions underreported AFP cases compared to the WHO surveillance target and may be at risk of not detecting an imported case of poliomyelitis. On the other hand, a number of subnational regions reported a higher number of cases than expected but were not likely to have been associated with an unidenti-

fied enterovirus cluster based on the available evidence, notwithstanding that stool specimens were not collected from more than half the cases.

Australia has never met the strict WHO surveillance target for adequate stool collection from 80% of the non-polio AFP cases; however 69% of the cases had at least one specimen collected within 14 days of the onset of paralysis. Enterovirus surveillance acts as a supplement to the AFP surveillance program in Australia, providing an additional means of monitoring Australia's polio-free status. In 2015, the WHO Regional Office for Europe released *Enterovirus surveillance guidelines*, which covered the rationale and characteristics of an ideal enterovirus surveillance system in support of the polio eradication initiative.²⁰ The guidelines included the protocol for the semi-nested RT-PCR amplification of enterovirus RNA that is the basis of the NERL's enterovirus typing procedure.

The worldwide removal of the Sabin poliovirus type 2 strain from the oral polio vaccine, in April 2016, was a historic event in vaccinology and public health.⁹ This event was based on the certification of wild poliovirus type 2 eradication in 2015 and the need to stop outbreaks of circulating vaccine-derived poliovirus type 2 from occurring in areas with low polio vaccine coverage. The switch from trivalent to bivalent oral polio vaccine, containing Sabin poliovirus types 1 and 3 strains, is the start of the polio endgame with a focus on the laboratory containment of poliovirus stocks and poliovirus potentially infectious material.⁸ The initial phase involved the destruction of wild and vaccine strains of poliovirus type 2 or transfer to a designated polio essential facility, such as the NERL.

Table 4: Enterovirus test results from the NERL Australia, 1995 to 2016.

Year	Poliovirus		Non-polio enterovirus	No enterovirus detected	EVID results referred ^a	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 ^b	60	1	9	9	0	79
2000	45	0	44	47	0	136
2001 ^b	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 ^c	0	2	32	115	107	256
2008	0	0	20	92	77	189
2009 ^d	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
2013 ^e	1	0	242	198	230	671
2014	0	0	68	128	506	702
2015 ^f	12	0	185	96	168	461
2016	0	0	242	143	227	612

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

b Untyped enterovirus or uncharacterised poliovirus isolates were referred for further testing after completion of a laboratory inventory. The 6 isolates (one in 1999 and five in 2001) tested as non-Sabin-like and were subsequently identified as wild type poliovirus prototype strains and were destroyed.

c Wild poliovirus type 1 was imported from Pakistan.

d A Sabin-like poliovirus type 1 was identified from an unimmunised infant.

e A Sabin-like poliovirus type 2 was identified from an infant who was immunised overseas with oral polio vaccine and hospitalised with diarrhoea upon return to Australia.

f Ten archived Sabin-like poliovirus type 1 samples were identified during a laboratory clean-up. Single isolations of Sabin-like poliovirus type 2 and type 3 were identified from sewage.

The detection of Sabin poliovirus type 2 in sewage in India in August–September 2016 led to a major public health investigation that identified the source as a number of private clinics continuing to administer trivalent oral polio vaccine after the global cessation date.²¹ This demonstrated the importance of polio containment surveys, such as was completed in Australia for wild poliovirus strains in 2002, and also the sensitivity of environmental surveillance to detect all types of poliovirus.^{22,23} The number of sewage collection sites has increased in polio-endemic countries and WHO plans to expand environmental surveillance in polio-free countries and especially those at high risk of polio importation. The NERL previously tested sewage collected in regional New South Wales and metropolitan Melbourne between 2010 and 2015 and will resume regular environmental testing in 2017.

Nigeria was declared polio-free in September 2015, after 12 months without detection of any wild poliovirus. The subsequent reporting of four polio cases in Nigeria due to indigenous (not imported) wild poliovirus type 1 in August 2016 was a setback but also a significant reminder of the need to maintain sensitive AFP surveillance and stool specimen collection.⁵ Nevertheless the reporting of 37 cases of wild poliomyelitis, all due to type 1, in Afghanistan, Nigeria and Pakistan represents the lowest number of polio cases reported since the eradication initiative commenced in 1988. In 2016, WHO maintained the declaration that the international spread of poliovirus continued to be a public health emergency of international concern and placed public health conditions on the countries involved to limit the global transmission of wild poliovirus and vaccine-derived poliovirus.¹¹

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