

# AUSTRALIAN NATIONAL ENTEROVIRUS REFERENCE LABORATORY ANNUAL REPORT, 2014

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## Abstract

Following the World Health Organization (WHO) recommendation, Australia conducts surveillance for cases of acute flaccid paralysis (AFP) in children less than 15 years of age as the main method to monitor its polio-free status. 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 2014, no cases of poliomyelitis were reported from clinical surveillance and Australia reported 1.4 non-polio AFP cases per 100,000 children, meeting the WHO performance criterion for a sensitive surveillance system. Non-polio enteroviruses can also be associated with AFP and enterovirus A71 and echovirus types 6 and 7 were identified from clinical specimens from cases of AFP. Globally, 359 cases of polio were reported in 2014, with the 3 endemic countries, Afghanistan, Nigeria and Pakistan, accounting for 95% of the cases. In May 2014, the WHO declared the international spread of wild poliovirus to be a public health emergency of international concern and has since maintained recommendations for polio vaccination of travellers from countries reporting cases of wild polio. *Commun Dis Intell* 2017;41(2):E161–E169.

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 5 sentinel tertiary paediatric hospitals.<sup>1–3</sup> The WHO recommends that 2 faecal specimens be collected at least 24 hours apart and within 14 days of the onset of paralysis from cases of AFP for virological investigation to exclude poliovirus as the causative agent. It is a require-

ment 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 are reviewed by the Polio Expert Panel (PEP) and reported to the WHO as evidence of Australia's continued polio-free status.

Clinical specimen 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, 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 to 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. In 2014, the testing of environmental samples commenced at a sentinel site in metropolitan Melbourne.

The number of wild polio cases worldwide decreased from 416 in 2013 to 359 in 2014.<sup>4</sup> Pakistan reported 306 of the cases and has become the major source of wild poliovirus transmission, this also being the source of cases in neighbouring Afghanistan. Nigeria is the 3rd remaining endemic country with only 6 wild polio cases reported there in 2014, the lowest number since the global polio eradication program started in 1988. Only wild poliovirus serotype 1 was detected in 2014, with the last report of wild poliovirus type 3 being in Nigeria in November 2012 and of wild poliovirus type 2 being in India in 1999.<sup>4,5</sup> This latter achievement led to the removal of the Sabin 2 serotype from oral polio vaccine (a live vaccine) along with laboratory containment of this serotype, involving restricted access at a limited number of facilities worldwide in 2016.<sup>6,7</sup> All 3 serotypes will still be incorporated in the inactivated polio vaccine. In May 2014, the WHO Director-General declared

the international spread of wild poliovirus in the Northern Hemisphere low season to be a Public Health Emergency of International Concern. The situation has been assessed every 3 months since then and countries known to be exporting wild poliovirus must ensure all residents and long-term visitors are vaccinated between 4 weeks and 12 months prior to international travel.<sup>8,9</sup> The Australian Government updated the Poliomyelitis Outbreak Response Plan in December 2014. The plan will be activated in the event of a polio importation.<sup>10</sup>

This report summarises the polio surveillance program in Australia for 2014; 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 (telephone 03-9342 9607, email [enterovirus@mh.org.au](mailto:enterovirus@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.

According to the WHO surveillance criterion, to be classified as adequate, 2 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. The faecal specimens are tested free of charge by the NERL.

The PEP, a subcommittee of the Communicable Diseases Network 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-Barré 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;

- 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 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](http://www.who.int/wer/en/) (<http://www.who.int/wer/en/>). Ineligible cases are not reported to the 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. For Australia in 2014, this equated to 44 cases per year, based on the Australian Bureau of Statistics data released in December 2013. 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.

At the end of each calendar year, a number of AFP notifications remain pending as there is insufficient clinical and laboratory data for the PEP to report a final classification. The PEP classifies such notifications as 'polio compatible-zero evidence' if a final review reveals no proof of clustering among the cases. The WHO considers these cases to be non-polio AFP when determining Australia's case rate.

### 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).<sup>11,12</sup> Diagnostic laboratories in Australia are encouraged to refer enteroviruses of unknown serotype to the NERL for further characterisation.

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 VDPV, in a process known as intratypic differentiation (ITD).<sup>13</sup> 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.<sup>14</sup>

### Enterovirus surveillance

The ERLNA was established primarily as a means of detecting imported poliovirus among untyped enteroviruses from clinical specimens. The network consists of 11 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 (Flinders Medical Centre and the Institute of Medical and Veterinary Science), Tasmania (Royal Hobart Hospital), Victoria (Royal Children's Hospital and VIDRL) and Western Australia (Queen Elizabeth II Medical Centre and the Princess Margaret Hospital for Children).

The NERL encourages members of the ERLNA to perform their own enterovirus typing and report the results to the NERL for inclusion in the national enterovirus database. It has advised members of 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 4 laboratories for typing on a regular basis.

The NERL screens clinical specimens for enterovirus using a semi-nested RT-PCR directed to highly conserved sequence in the 5' non-translated region.<sup>15</sup> Enterovirus typing is primarily performed by amplifying a fragment of the VP1 genomic region according to a published method,<sup>16</sup> 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<sup>17</sup> and further advice was obtained from the Enterovirus Laboratory at the National Public Health Institute, Finland, a Global Specialised Laboratory in the WHO Polio Laboratory Network. In brief, 800 ml of sewage is collected as a grab sample prior to any biological or chemical treatment and referred to the NERL within 24 hours. At the laboratory, 500 mL of the sample is 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 collected the next day and clarified with chloroform as for a faecal specimen. Aliquots of the sewage concentrate are 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 utilised to confirm the cell culture results, as not all human enteroviruses infect the RD-A cell line. All enterovirus isolates from cell culture and positive detections by RT-PCR are investigated to determine the virus type by nucleic acid sequencing.

## Results

### Classification of acute flaccid paralysis cases

A total of 80 notifications of AFP cases involving children less than 15 years of age were received in 2014 (Table 1). The PEP classified 58 cases as non-polio AFP, a rate of 1.4 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 1 case of non-polio AFP per 100,000 children (Table 2, Figure 1). Twelve cases were notified by more than 1 source, whether by 2 or more clinicians or a clinician and the PAEDS system. Seven notifications were deemed to be ineligible due to the patient's age being greater than 14 years or where the clinical presentation was subsequently determined not to be AFP. Specimens were received from 2 notifications of AFP cases that were later classified by the PEP as polio compatible-zero evidence due to insufficient clinical information. The WHO accepts such cases as non-polio AFP when reviewing Australia's surveillance data due to the quality and breadth of the national polio surveillance program.

In 2014, an Australian adult was hospitalised upon returning from the Horn of Africa. The patient had experienced fever and headache and

**Table 1: Notification of acute flaccid paralysis cases, Australia, 2014, by state or territory**

State or territory	Estimated population aged <15 years*	Expected number of reported AFP cases in 2014	Total number of notifications	Ineligible notifications	Duplicate notifications	Polio compatible-zero evidence	Eligible cases with final classification by the Polio Expert Panel	Non-polio AFP rate per 100,000 children
ACT	70,739	1.0	0	0	0	0	0	0.0
NSW	1,398,648	14.0	17	3	1	0	13	0.9
NT	53,589	0.5	1	0	0	1	0	2.0
Qld	924,703	9.0	16	1	3	0	12	1.3
SA	295,303	3.0	3	1	0	0	2	0.7
Tas.	94,959	1.0	0	0	0	0	0	0.0
Vic.	1,051,114	10.5	35	2	7	1	25	2.5
WA	482,308	5.0	7	0	1	0	6	1.2
Australia	4,371,363	44.0	79	7	12	2	58	1.4

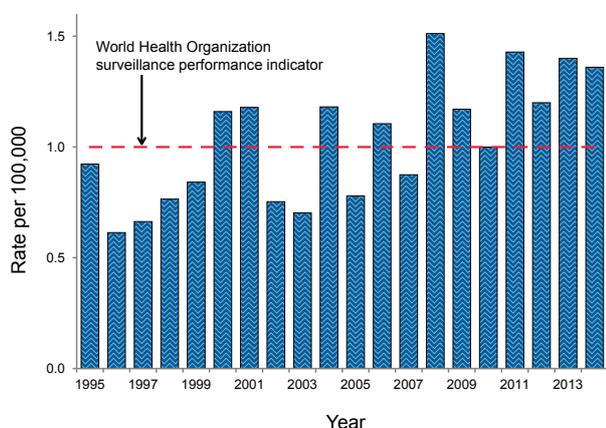
\* Australian Bureau of Statistics, estimated population at 30 June 2013. Available from the [Australian Bureau of Statistics web site \(www.abs.gov.au\)](http://www.abs.gov.au).

AFP Acute flaccid paralysis

**Table 2: Australia’s surveillance for cases of acute flaccid paralysis, 2014, compared with the main World Health Organization performance indicators**

WHO surveillance performance indicator for acute flaccid paralysis cases in children <15 years	Performance of Australia’s acute flaccid paralysis surveillance	
≥1.0 non-polio acute flaccid paralysis (AFP) case per 100,000 children (44 cases for Australia in 2014)	60 cases classified as non-polio AFP	1.36 (60 / 44) non-polio AFP cases per 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)	30 AFP cases with adequate specimens collected	50% (30 / 60) classified non-polio AFP cases with adequate specimens

**Figure 1: Non-polio acute flaccid paralysis rate for children less than 15 years of age, Australia, 1995 to 2014\***



\* The World Health Organization acute flaccid paralysis surveillance performance indicator for a polio non-endemic country is one case per 100,000 children less than 15 years of age, which is highlighted by the dotted line.

subsequently developed a flaccid paralysis of an upper limb while overseas. The attending clinicians in Australia notified the case as suspected poliomyelitis but enterovirus was not detected in the specimens referred to the NERL for testing. The case was initially reported to WHO as polio compatible pending further clinical and laboratory investigation. The differential diagnosis included acute myelitis due to viral infection but the risk to persons in contact with the index case while in transit and within Australia was considered very low based on enterovirus not being detected in the patient’s specimens. Polio serology was performed when acute and convalescent sera became available and no evidence of an increasing titre (that would be consistent with recent poliovirus infection) was observed. The case was subsequently classified by the PEP as non-polio AFP and diagnosed as West Nile virus infection by the attending clinicians based on serial serum sampling.<sup>18</sup>

**Notification of acute flaccid paralysis cases by state and territory**

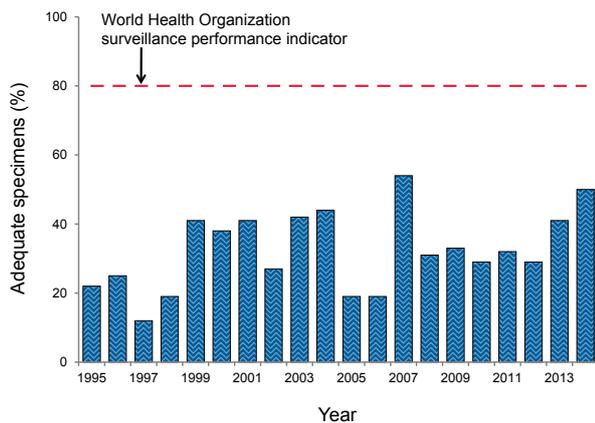
In 2014, AFP cases were notified from all jurisdictions in Australia except the Australian Capital Territory and Tasmania; it is estimated that these 2 jurisdictions will report 1 case per year based on the population less than 15 years of age so this result may not be surprising (Table 1). The non-polio AFP rates for eligible cases per jurisdiction exceeded the WHO AFP surveillance performance indicator of 1 case per 100,000 children in the Northern Territory, Queensland, Victoria and Western Australia, accounting for 73% of the national cases. The most populous state, New South Wales, did not achieve the expected rate of reporting of non-polio AFP cases for the first time since 2007.

**Faecal collection from acute flaccid paralysis cases**

A total of 95 faecal specimens from 47 of the 60 eligible cases were tested at the NERL in 2014, but only 30 AFP cases met the WHO criteria for adequate specimen collection with 2 specimens collected within 14 days of the onset of paralysis and more than 24 hours apart (Figure 2, Tables 2 and 3). The proportion of cases with at least 1 specimen collected within 14 days of the onset of paralysis was 73%, while 78% of cases had a specimen collected any time after the onset of paralysis. No poliovirus was detected in any of the specimens. The NPEVs echovirus types 6 and 7 were individually detected from faecal specimens of 2 AFP cases. Enterovirus A71 was detected from 1 AFP case with onset in December 2014, which was in contrast to the outbreak reported in 2013, when this type of NPEV was reported from 9 AFP cases between February and September.

**Table 3: Specimens referred to the National Enterovirus Reference Laboratory, Australia, 2014**

Result	Specimens from acute flaccid paralysis cases involving children <15 years of age	Specimens from acute flaccid paralysis cases involving patients ≥15 years of age	Enterovirus surveillance	Environmental surveillance	Total
Non-polio enterovirus	3	0	62	3	68
Rhinovirus	0	0	2	0	2
No enterovirus identified	92	5	31	0	128
Total	95	5	95	3	198

**Figure 2: Adequate faecal specimen rate, Australia, 1995 to 2014\***

\* The main World Health Organization criteria for adequate specimen collection is 2 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 acute flaccid paralysis.

### Enterovirus surveillance

Poliovirus was not detected by any member of the ERLNA in 2014, with a total of 506 NPEVs typed by members of the network from clinical specimens (Table 4). The most common genotypes identified in order of decreasing frequency were coxsackievirus A6, echovirus 6, coxsackievirus B5 and coxsackievirus B4 accounting for two-thirds of the total, while only sporadic detections of enterovirus A71 were reported.

### Environmental surveillance

Environmental surveillance for poliovirus was established at a sentinel site in metropolitan Melbourne from December 2014. No poliovirus was identified from the 3 grab samples tested but each was positive for NPEVs that act as an indicator organism for the collection, transport and test procedures. It is planned to continue the collections at the Melbourne site on a weekly basis through 2015 and then reassess the choice of site based on the results.

### Poliovirus regional reference laboratory activities

In 2014, as part of its role as a Polio Regional Reference Laboratory, the NERL received specimens from AFP cases referred from Brunei Darussalam (2 cases), Pacific Island countries (12 cases) and Papua New Guinea (10 cases). The Sabin poliovirus serotype 3 vaccine strain was isolated from both specimens of 1 AFP case in Papua New Guinea, along with an NPEV. NPEVs were reported from another 7 cases in Papua New Guinea and 4 AFP cases from the Pacific Islands.

### Quality assurance programs

In 2014, the NERL was accredited as a WHO Polio Regional Reference Laboratory through participation in the annual WHO polio quality assurance panels for RT-PCR for ITD and vaccine derived poliovirus and poliovirus VP1 sequencing. The laboratory also successfully participated in the Royal College of Pathologists of Australasia quality assurance panel for enterovirus detection by RT-PCR.

### Discussion

In 2014, Australia reached the WHO surveillance target of  $\geq 1$  non-polio AFP case per 100,000 children under 15 years of age, for the 7th year in a row. The combination of clinicians notifying AFP cases via the APSU monthly report card and nurses ascertaining cases through the PAEDS system provided Australia with a polio surveillance system that meets the international standard to detect an imported case of polio in children less than 15 years of age through these well-established schemes.<sup>1-3</sup> Australia has never met the strict WHO surveillance target for adequate stool collection from 80% of the non-polio AFP cases, with 47% of cases having 2 specimens tested in 2014, and 65% of cases with at least 1 specimen.

Three different types of NPEV-echovirus types 6 and 7 and enterovirus A71 were detected from 3 distinct AFP cases in 2014. Since most enterovi-

**Table 4: Enterovirus test results from the National Enterovirus Reference Laboratory, Australia, 1995 to 2014**

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 <sup>†</sup>	60	1	9	9	0	79
2000	45	0	44	47	0	136
2001 <sup>†</sup>	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 <sup>‡</sup>	0	2	32	115	107	256
2008	0	0	20	92	77	189
2009 <sup>§</sup>	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 <sup>¶</sup>	1	0	242	198	230	671
2014	0	0	68	128	506	702

\* Enterovirus Identification (EVID) results include retrospective data made available via the Enterovirus Reference Laboratory Network of Australia.

† Untyped enterovirus or uncharacterised poliovirus isolates were referred for further testing after completion of a laboratory inventory. The 6 isolates tested as non-Sabin-like and were subsequently identified as wild type poliovirus prototype strains and were destroyed.

‡ Wild poliovirus type 1 was imported from Pakistan.

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

¶ 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.

rus infections are asymptomatic, and the viruses were detected from a non-sterile site (faeces), the results could be an incidental finding; however, all three viruses have been associated with AFP worldwide. Even though enterovirus A71 was reported from only one AFP case in 2014, public health officials continue to monitor accounts of this enterovirus type after the outbreak in 2013, when it was detected from 9 AFP cases in New South Wales and Victoria.

Enterovirus D68 became of public health interest in 2014, with reports of geographical and temporal associations with AFP in the United States of America (USA).<sup>19</sup> Enterovirus D68 was first isolated in 1962 and its detection in clinical specimens worldwide was sporadic until 2008, when acute respiratory illness including fatalities were reported in the Philippines (2008–2009), United Kingdom (2009–2010), Japan (2010),

Netherlands (2010), New Zealand (2010), Canada (2014) and the USA (2014).<sup>19,20</sup> While enterovirus D68 was not detected in cerebrospinal fluid during the USA outbreak, there have been 2 such reports: a young adult with AFP and a fatal meningomyeloencephalitis in a 5-year-old in 2005 and 2008, respectively.<sup>21,22</sup> The extensive clinical, virological and epidemiological evidence from the enterovirus D68 outbreak during 2014 in the USA strengthens the evidence for a causal association with AFP but further investigation of the virus lineage to elucidate the genetic elements concerned with neurological disease is warranted. Enterovirus D68 was detected once from an AFP case in Australia in 2010<sup>23</sup> and heightened activity was noted in Western Australia in 2011 and 2013.<sup>24</sup> The NERL will continue to screen the ERLNA typing results for any apparent variation in enterovirus transmission of this and other enterovirus types.

Environmental surveillance by testing grab samples of raw sewage has proved to be a sensitive means of detecting poliovirus in the absence of clinical cases overseas, such as occurred in Israel with 150 positive samples in 2013 to 2014<sup>25</sup> and in Quetta, Rawalpindi and Lahore in Pakistan in 2014.<sup>26</sup> WHO estimates the environmental test protocol to be capable of detecting 1 person shedding poliovirus among 10,000 uninfected persons.<sup>17</sup> Australia implemented sentinel environmental surveillance for poliovirus at 4 rural and regional sites from 2009. In 2012, the Polio Surveillance Systems Review recommended environmental surveillance be trialled at a major metropolitan site<sup>27</sup> and this led to testing commencing in Melbourne in 2014. The much larger population catchment of a metropolitan site necessitated more frequent testing to compensate for reduced sample sensitivity compared with the quarterly testing at the rural and regional sites, so collections in Melbourne will continue on a weekly basis in 2015.

It is extremely important that all countries employ sensitive surveillance for AFP in children and poliovirus even as the global case count and number of endemic countries reduces. It will require 3 years of sensitive surveillance to certify global polio eradication due to most infections being asymptomatic and to account for potential gaps in immunisation and surveillance.

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