Australian Government
Department of Health and Aged Care

2023 · Volume 47

Communicable Diseases Intelligence

Australian National Enterovirus Reference Laboratory annual report, 2022

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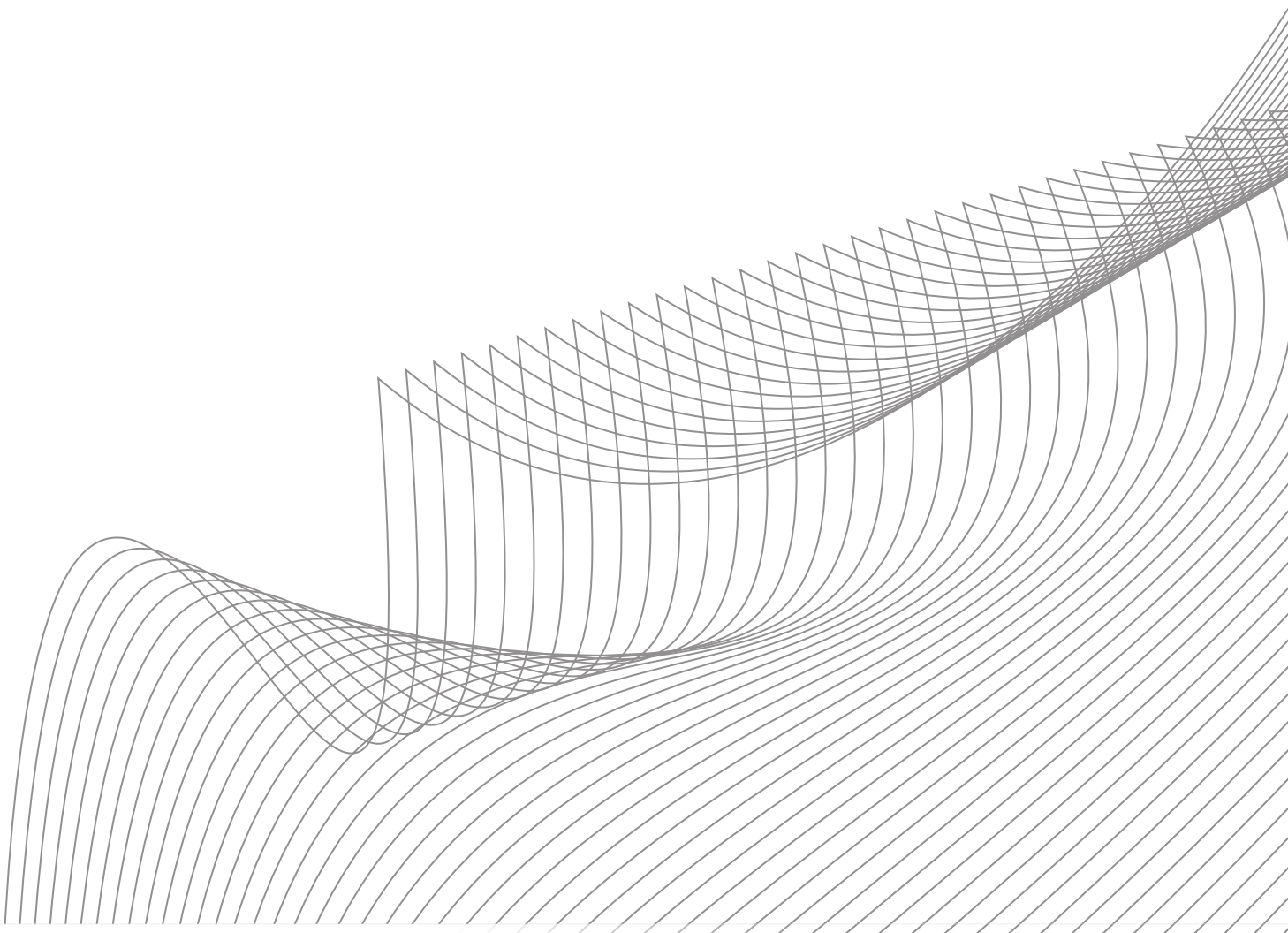

https://doi.org/10.33321/cdi.2023.47.35
Electronic publication date: 26/06/2023
http://health.gov.au/cdi

Communicable Diseases Intelligence
ISSN: 2209-6051 Online

This journal is indexed by Index Medicus and Medline.

Communicable Diseases Network Australia
Communicable Diseases Intelligence contributes to the work of the Communicable Diseases Network Australia.
http://www.health.gov.au/cdna

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Website
http://www.health.gov.au/cdi

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Annual report

Australian National Enterovirus Reference Laboratory annual report, 2022

Matthew B Kaye, Linda K Hobday, Aishah Ibrahim, Leesa Bruggink, Bruce R Thorley

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 2022, no cases of poliomyelitis were reported from clinical surveillance and Australia reported 1.69 non-polio AFP cases per 100,000 children, thereby meeting the WHO's performance criterion for a sensitive surveillance system. The non-polio enteroviruses coxsackievirus A2, coxsackievirus A6, coxsackievirus A10, echovirus 18, enterovirus A71 and enterovirus C96 were identified from clinical specimens collected from AFP cases. Australia also performs enterovirus and environmental surveillance to complement the clinical system focussed on children. In 2022, thirty cases of wild poliovirus were reported from three countries (Afghanistan, Mozambique and Pakistan); 24 countries also reported cases of poliomyelitis due to circulating vaccine-derived poliovirus.

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

Introduction

Poliomyelitis (polio) is caused by the three poliovirus types 1, 2 and 3. Approximately 90% of wild poliovirus infections are asymptomatic or produce a non-specific fever. Paralysis occurs in fewer than 1% of poliovirus infections, with a further 1% resulting in aseptic meningitis; the remainder of symptomatic infections exhibit fever, headache, malaise, nausea and vomiting.[1] Polio evolved during the 19th and 20th centuries to become a global disease with annual epidemics, until the development of the inactivated (Salk) and live attenuated (Sabin) poliovirus vaccines in the 1950s and 1960s.[2] Since 1988, when the World Health Assembly declared the goal of global polio eradication, an estimated 20 million cases of paralytic polio have been avoided and 1.5 million lives saved.[3]

In 2000, the World Health Organization's (WHO) Western Pacific Region, which includes Australia, was declared polio-free.[4] Australia has established clinical and virological surveillance systems to monitor its polio-free status. The clinical surveillance program follows the WHO recommendation of investigating acute flaccid paralysis (AFP) cases in children less than 15 years of age due to a higher risk of poliovirus infection. Cases of AFP are ascertained either by clinicians notifying the Australian Paediatric Surveillance Unit (APSU), or through the Paediatric Active Enhanced Disease Surveillance System (PAEDS) at eight sentinel tertiary paediatric hospitals.[5,6] The WHO recommends two faecal specimens be collected for virological investigation more than 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), at the Peter Doherty Institute for Infection and Immunity. The clinical and laboratory data from AFP cases in children are reviewed by the Polio Expert Panel (PEP) and are reported to the WHO as evidence of Australia's continued polio-free status.

Enterovirus and environmental surveillance programs were established in Australia as virological surveillance for poliovirus, to complement the clinical surveillance program focussed on AFP cases in children. Non-polio enteroviruses, such as enterovirus A71 (EV-A71) and enterovirus D68 (EV-D68), have been associated with AFP, with an increased interest in the latter after reports of a possible association with acute flaccid myelitis since 2010.[7,8] Non-paralytic poliovirus infection may manifest clinically from a mild febrile illness to meningitis or meningoencephalitis. 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 in Australia. Most poliovirus infections are asymptomatic, with the virus shed for weeks in the faeces of infected persons. The WHO recognises the testing of environmental samples, such as raw sewage and river water, as a means of detecting the presence of wild poliovirus and vaccine-derived poliovirus (VDPV) in polio-free countries.

While 2021 saw the lowest number of wild poliovirus cases ever recorded, the number of cases worldwide increased from six in 2021 to 30 in 2022, with wild poliovirus type 1 (WPV1) detected in Afghanistan (n = 2), Mozambique (n = 8) and Pakistan (n = 20).[9] Global eradication of wild poliovirus types 2 and 3 was certified in 2015 and 2019 respectively.[10] Although WPV1 circulation has remained endemic in Afghanistan and Pakistan, the detection of WPV1 in Mozambique is a setback following the August 2020 certification of the WHO African Region as wild-poliovirus-free, four years after the last case of WPV1 was reported in Nigeria.[11] In 2022, nine cases of WPV1 were reported in Africa: the first was a child from Malawi, who developed AFP in November 2021 and was consequently recorded as a 2021 case; the second was a child from Mozambique who developed AFP in March 2022 and who preceded the detection of a further seven cases in Mozambique.[9,12] In both countries, the virus is genetically linked to a virus circulating in the Sindh province in Pakistan in 2019, highlighting that poliovirus anywhere is a risk to people everywhere.[12,13]

Polio outbreaks due to circulating VDPV (cVDPV) can emerge in areas where poor sanitation standards occur in conjunction with sustained low oral poliomyelitis vaccine (OPV) coverage. cVDPV continues to present a challenge for the global polio eradication program, with the number of related AFP cases increasing in 2022 compared to 2021.[14] Indeed, cVDPV was detected in 814 AFP cases and 437 environmental samples in 2022, with detections across 32 countries, 27 of which were in the WHO African and Eastern Mediterranean Regions.[14] However, in July 2022, a case of poliomyelitis related to cVDPV type 2 (cVDPV2) was confirmed in an unvaccinated adult from Rockland County, New York, marking the first case of poliomyelitis reported in the United States of America since 2013.[15,16] Related virus was subsequently detected in 94 environmental samples collected in New York, as well as in environmental samples collected in London and Canada, and is genetically linked to viruses detected in sewage samples collected between April and October 2022 from Jerusalem District, Israel.[16-18] The recurrence of poliovirus in well-resourced, developed countries highlights the crucial need to maintain high levels of polio vaccine coverage and sensitive polio surveillance systems until the global eradication of poliovirus has been certified.

This report summarises the poliovirus surveillance program in Australia for 2022, encompassing clinical surveillance for AFP cases in children and virological surveillance for poliovirus.

Methods

Acute flaccid paralysis surveillance

Poliovirus infection, including suspected poliomyelitis, is notifiable under the National Notifiable Diseases Surveillance System.[19] For AFP cases involving children less than 15 years of age, paediatricians are requested to notify the NERL directly,[i] and to complete a clinical questionnaire.[ii,5] Designated nursing staff ascertain AFP cases from the medical records at the eight tertiary paediatric hospitals where PAEDS operates.[6] Duplicate notifications of AFP cases from both paediatricians and PAEDS staff can occur; such duplication reflects a sensitive surveillance system. While clinical information from more than one source is utilised by the PEP, duplicate notifications are excluded from data analyses.

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.[20] The faecal specimens are tested by virus culture at the NERL with funding from the Australian Government Department of Health and Aged Care.

The PEP, a subcommittee of the Communicable Diseases 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-Barré syndrome and transverse myelitis) or an Australian of any age with suspected polio.

The PEP classifies cases of AFP as:

1. Poliomyelitis due to wild poliovirus, VDPV, or vaccine associated paralytic poliomyelitis (VAPP);

2. Polio compatible if there is insufficient evidence to exclude poliomyelitis;

3. Non-polio AFP; or

4. 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 the WHO for inclusion in the global AFP surveillance data published in the Weekly Epidemiological Record.[iii] Ineligible cases are not reported to the WHO.

The WHO annual AFP surveillance performance indicator target for a polio non-endemic country is at least one case of non-polio AFP per 100,000 children aged less than 15 years.[20] The target non-polio AFP rate is calculated by dividing the number of children less than 15 years of age by 100,000 and rounding to a whole number, which for Australia in 2022 equated to 48 cases based on the Australian Bureau of Statistics estimate of Australia's population at 30 June 2021. 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. An AFP surveillance scheme that meets the WHO surveillance performance indicators is considered sensitive enough to detect the importation of wild poliovirus or cVDPV in a polio-free country.

i Telephone: 03 9342 9607; email: enterovirus@vidrl.org.au.

ii Available online at https://my.fuzee.com/apsu-vidrl/afpquestionnaire.html.

iii Available online at http://www.who.int/wer/en/.

Virus culture

Faecal specimens are treated with minimum essential medium containing Earle's salts and extracted with chloroform, which enteroviruses are resistant to, for removal of bacteria and fungi. The suspension is clarified via centrifugation and the supernatant inoculated onto the two mammalian cell lines recommended by the WHO for the isolation of poliovirus: L20B (a transgenic mouse epithelial cell line expressing the human poliovirus receptor, CD155) and RD-A (human rhabdomyosarcoma).[21,22] Inoculated cell cultures are observed microscopically, for between seven and 14 days, for the presence of cytopathic effects that indicate likely infection with a poliovirus (L20B-positive cultures) or with a non-polio enterovirus (RD-A-only positive cultures). All enterovirus isolates from cell culture are typed by nucleic acid sequencing as described in the "Enterovirus surveillance" section below.

Reverse-transcription polymerase chain reaction

L20B-positive cell cultures are tested by two WHO reverse transcription real-time polymerase chain reaction (RT-qPCR) assays used to determine whether the cultured isolate is a non-polio enterovirus, a wild poliovirus, an OPV strain, or a VDPV, in a process known as intratypic differentiation (ITD).[23] The NERL sequences the complete poliovirus viral protein 1 (VP1) genomic region of all polioviruses. The genomic sequence of the VP1 region, which contains a major neutralising antibody binding site, provides valuable biological information, including the number of mutations within a significant region of OPV virus strains, and it enables phylogenetic analysis of wild poliovirus so as to rapidly determine the likely source of the virus, as utilised in the 2007 case of a wild poliovirus importation into Australia.[24]

Environmental surveillance

Environmental surveillance was initially established in regional New South Wales in 2010. Since 2014, testing has focussed on metropolitan Melbourne, with sewage samples collected from both the Eastern and Western Treatment Plants. In 2022, environmental surveillance was expanded to include testing of sewage samples collected from wastewater treatment plants in metropolitan Perth (Beenyup, Subiaco and Woodman Point), in addition to the samples collected in Melbourne. Environmental samples are processed by the NERL according to the two-phase separation procedure published by the WHO.[25] In brief, 800 ml of sewage is collected as a grab sample prior to any biological or chemical treatment. 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 using chloroform treatment and centrifugation. The sample extract is inoculated onto L20B and RD-A cell lines and observed microscopically for cytopathic effect as for faecal specimens.

Enterovirus surveillance

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

Although the NERL encourages members of the ERLNA to perform their own enterovirus typing, several laboratories continue to refer un-typed enteroviruses to the NERL for typing. Further, the network is a voluntary and passive system, such that laboratory participation and the number of results or referred specimens received by the NERL varies from year to year.

Clinical specimens are initially screened for enterovirus using a RT-qPCR assay directed to highly conserved genomic sequence in the 5' untranslated region (UTR).[26] Enterovirus typing is performed on enterovirus-positive samples using an in-house nested RT-PCR assay: the first round of the assay amplifies the entire capsid-encoding region of the virus and the second round targets a fragment of the VP1 genomic region. If the typing assay does not amplify a suitable fragment for sequencing and type determination, a second, semi-nested RT-PCR assay that targets a fragment of the 5'UTR is used to characterise the enterovirus to the level of *Enterovirus* species only, and may be used to exclude the presence of poliovirus.

Results

Classification of AFP cases

In 2022, a total of 96 notifications of AFP cases were received (Table 1). Of these, 15 notifications were reported by the APSU surveillance system and 81 through PAEDS. Three notifications were deemed to be ineligible as the clinical presentation was subsequently determined not to be AFP. Twelve notifications were duplicates; notified by more than one source, whether by two or more clinicians through the APSU or by a clinician and the PAEDS system.

The PEP classified 81 cases as non-polio AFP, a rate of 1.69 cases per 100,000 children less than 15 years of age, which met the WHO AFP surveillance performance criterion for a polio-free country of at least one case of non-polio AFP per 100,000 children (Table 2, Figure 1). This result, which marks the fifteenth consecutive

Table 1: Notification of acute flaccid paralysis cases, 2022 by state and territory

State or territory[a]	Estimated population aged < 15 years[b]	Expected number of AFP cases in 2022[c]	Total number of notifications	Ineligible notifications	Duplicate notifications	Eligible AFP cases with final classification by PEP	Non-polio AFP rate per 100,000 children[d]
ACT	82,770	1	1	0	0	1	1.00
NSW	1,512,737	15	31	1	2	28	1.87
NT	52,381	1	2	0	0	2	2.00
Qld	999,957	10	17	0	8	9	0.90
SA	309,214	3	6	0	1	5	1.67
Tas.	93,389	1	0	0	0	0	0.00
Vic.	1,200,896	12	31	1	1	29	2.42
WA	520,584	5	8	1	0	7	1.40
Australia	**4,771,928**	**48**	**96**	**3**	**12**	**81**	**1.69**

a ACT: Australian Capital Territory; NSW: New South Wales; NT: Northern Territory; Qld: Queensland; SA: South Australia; Tas.: Tasmania; Vic.: Victoria; WA: Western Australia.

b Australian Bureau of Statistics, estimated population at 30 June 2021. Available at www.abs.gov.au.

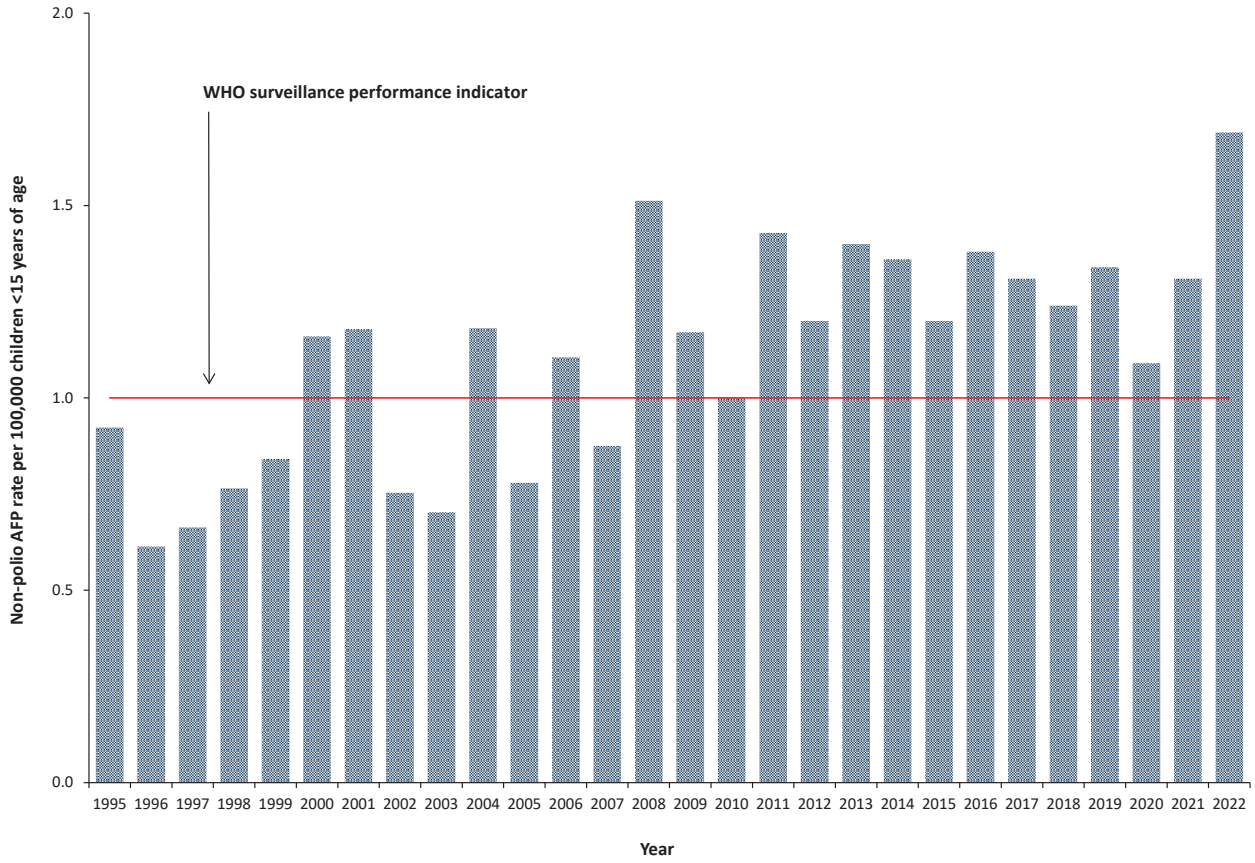
c The expected number of AFP cases for Australia is calculated by dividing the estimated population < 15 years of age by 100,000 and rounding to a whole number.

d The non-polio AFP rate is calculated by dividing the number of eligible PEP-classified AFP cases by the number of expected AFP cases.

Table 2: Australia's surveillance for cases of acute flaccid paralysis, 2022, 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 per 100,000 children (48 cases for Australia in 2022)	81 cases classified as non-polio AFP	1.69 (81 / 48) non-polio AFP cases per 100,000 children <15 years
≥ 80% of classified AFP cases with adequate specimens (two faecal specimens collected more than 24 hours apart and within 14 days of onset of paralysis)	58 AFP cases with adequate specimens collected	72% (58 / 81) classified non-polio AFP cases with adequate specimens

Figure 1: Non-polio acute flaccid paralysis rate, Australia 1995 to 2022[a]



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

year in which Australia has achieved the WHO AFP surveillance target, is the highest non-polio AFP rate ever achieved in Australia.

Of the 81 non-polio AFP cases: ten cases were notified by clinicians through both the APSU and PAEDS systems; 68 cases were notified through the PAEDS system only; and three cases were notified through the APSU system only. The three cases unique to the APSU system were notified by clinicians at hospitals where PAEDS does not operate, and therefore would not have otherwise been detected using the PAEDS system alone. Guillain-Barré syndrome and transverse myelitis were the most common causes of non-polio AFP in 2022, with the PEP

classifying 26 and 12 cases, respectively, with these two conditions. Eleven cases were classified as acute disseminated encephalomyelitis, three cases were classified as botulism and another three cases as tick bite paralysis.

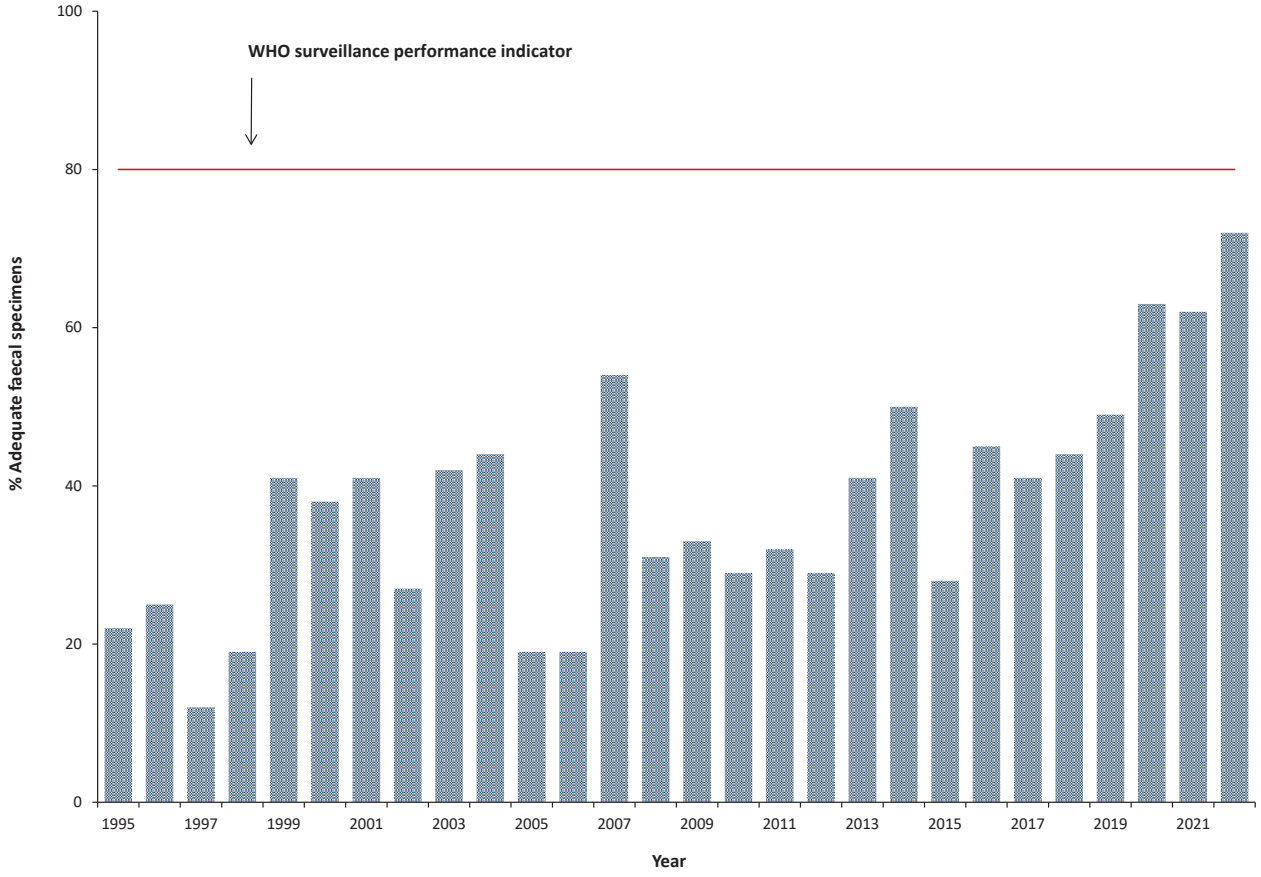
Notification of AFP cases by state and territory

In 2022, AFP cases were notified from all jurisdictions in Australia except Tasmania (Table 1). The non-polio AFP rates for eligible cases met the WHO AFP surveillance performance indicator of at least one case per 100,000 children less than 15 years of age in the Australian Capital Territory, New South Wales, Northern Territory, South Australia, Victoria and Western Australia, with Queensland and Tasmania the only jurisdictions not reaching the target.

Faecal collection from AFP cases

In 2022, a total of 155 faecal specimens from 78 of the 81 eligible cases were tested at the NERL. Two specimens were collected more than 24 hours apart and within 14 days of the onset of paralysis from 58 of the eligible cases, satisfying the WHO criterion for adequate specimens and representing 72% of the non-polio AFP cases compared to the WHO benchmark of 80% (Figure 2, Table 2). Although Australia has never attained this surveillance performance criterion, the percentage of adequate stools collected in 2020 (63%), 2021 (62%) and 2022 (72%) marks a significant improvement from previous years, in which the proportion of adequate stools was frequently less than 50%, and demonstrates a continuing improvement in this performance metric (Figure 2). While the optimal period to collect stool specimens is within 14 days of the onset of paralysis, poliovirus can be detected for up to 60 days after the

Figure 2: Adequate faecal specimen collection rate, Australia 1995 to 2022[a]



a The 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, which is indicated by the red line.

onset of paralysis; Eighty-four percent of cases (68/81) had two specimens collected within this extended time frame.[20]

Poliovirus was not detected in any of the specimens referred for AFP surveillance. Non-polio enteroviruses, including one each of coxsackievirus A2, coxsackievirus A6, coxsackievirus A10, echovirus 18, EV-A71, and enterovirus C96, were identified from stool specimens collected from six separate AFP cases. Three of these were from cases in New South Wales (coxsackievirus A6, coxsackievirus A10, EV-A71), one in the Northern Territory (enterovirus C96), one in Victoria (echovirus 18) and one in Western Australia (coxsackievirus A2). A further identification of EV-A71 was also reported in a separate AFP case from New South Wales, but only in a nasopharyngeal aspirate, not in the stool specimens from this case. Non-polio enteroviruses, identified from stool specimens collected from another five AFP cases could only be characterised as *Enterovirus* species due to low viral load: two from cases in New South Wales (*Enterovirus A* and *Enterovirus B*), one in Queensland (*Enterovirus A*) and two in Victoria (both *Enterovirus B*).

Environmental surveillance

In 2022, the NERL tested nine environmental samples. Eight of these samples were collected as part of the routine environmental surveillance programme, which includes monthly sample collections alternating between the Eastern and Western Treatment Plants in Melbourne (re-commenced August 2022), and a second monthly sample collection rotating between Beenyup, Subiaco and Woodman Point wastewater treatment plants in Perth (beginning October 2022). Poliovirus was not detected in any of these samples.

The ninth environmental sample in 2022 was referred by Sydney Water after detection of poliovirus in a wastewater sample collected on 8 December from the Quakers Hill sewage treatment plant. The NERL isolated poliovirus type 3 from this sample and the nucleotide sequence of the VP1 region had 99.8% identity to the prototype Sabin vaccine strain, indicative of a recent vaccination event.

Non-polio enteroviruses were isolated from all nine environmental samples tested, with species B enteroviruses, including coxsackievirus B5 and echovirus 11, the most common enterovirus detected, identified in eight of the nine (89%) environmental samples. The enterovirus type in the remaining sample could not be resolved due to mixed genomic sequence. Enterovirus infections are considered ubiquitous; the isolation of non-polio enteroviruses, from environmental samples collected in polio-free countries not using OPV, serves as an indicator of the quality of the sewage collection and test procedures.

Table 3: Laboratory results for Australian specimens reported by the NERL, 2022

Result	Specimens from AFP cases involving children < 15 years of age	Specimens from AFP cases involving patients ≥ 15 years of age	Environmental surveillance[a]	Enterovirus surveillance[b]	Total
Sabin poliovirus type 3	0	0	1	0	1
Rhinovirus	1	0	0	0	1
Non-polio enterovirus	26	0	9	173	208
No enterovirus identified	139	7	0	29	175
Total	**166**	**7**	**10**	**202**	**385**

a A total of nine environmental samples were tested, with a Sabin poliovirus type 3 and a non-polio enterovirus both detected in the same sample.

b A total of 207 specimens were referred for enterovirus typing, with five specimens being inadequate for testing.

Enterovirus surveillance

In 2022, a total of 207 clinical specimens were referred to the NERL for enterovirus typing (Table 3). One hundred and fifty-five specimens (74.9%) were referred from Victoria and 52 (25.1%) from interstate: six from the Australian Capital Territory; one from New South Wales; one from Queensland; 39 from South Australia; and five from Tasmania. Of these specimens, 173 (83.6%) were characterised as non-polio enteroviruses, with 129 (74.6%) being fully typed based on VP1 sequence and 44 (25.4%) characterised to the level of *Enterovirus* species based on 5'UTR sequence. Of the remaining specimens, 29 (14.0%) were reported as 'no enterovirus identified' and five (2.4%) were inadequate for testing (Table 3). Poliovirus was not detected in any of the specimens referred for enterovirus typing.

In 2022, including specimens received for AFP and environmental surveillance, a total of 148 non-polio enteroviruses were typed and an additional 60 enteroviruses were characterised to the level of *Enterovirus* species by the NERL (Table 3). Excluding rhinoviruses, a total of 384 enterovirus typing results were reviewed by the NERL, with no additional typing results referred from members of the ERLNA (Table 4). In order of decreasing frequency, the most common types of non-polio enteroviruses identified by the laboratory network in 2022 were coxsackievirus A6, echovirus 18, coxsackievirus B2, and echovirus 30, which together accounted for 70% (104/148) of all enteroviruses typed in 2022.

Three cases of EV-A71 and two cases of EV-D68 were also detected in 2022. Two of the EV-A71 cases (both from New South Wales) were identified through AFP surveillance, although for one of the cases the virus was only detected in a nasopharyngeal aspirate. The clinical presentation of these cases included encephalomyelitis and brainstem encephalitis. The third case of EV-A71 was identified through enterovirus surveillance from the cerebrospinal fluid of a patient in the Australian Capital Territory. Both cases of EV-D68 were identified through enterovirus surveillance: one from a faecal specimen from South Australia and the other from a nasopharyngeal swab from Victoria. The widespread distribution of these cases did not support a common transmission link for either virus.

Polio regional reference laboratory activities

In 2022, as part of its role as a Polio Regional Reference Laboratory, the NERL did not isolate poliovirus from any AFP cases. The laboratory received four stool specimens from two AFP cases in Brunei Darussalam and 13 stool specimens from seven AFP cases in Fiji. Coxsackievirus B2 was detected in two specimens from one AFP case from Fiji and an enterovirus characterised as *Enterovirus* species *C* based on 5'UTR sequence was detected in two specimens from a second AFP case from Fiji.

A total of 123 stool specimens were received from Papua New Guinea and tested by the NERL, including 121 from AFP cases involving children less than 15 years of age and two from contacts of AFP cases. Non-polio enteroviruses were detected in 51.2% (63/123) of the specimens, with coxsackievirus A24 and enterovirus C99 the most common enteroviruses detected.

Quality assurance programs

In 2022, the NERL maintained its accreditation as a WHO Polio Regional Reference Laboratory through the successful completion of annual WHO quality assurance panels for poliovirus isolation, poliovirus ITD and poliovirus sequencing. The NERL also successfully participated in the Royal College of Pathologists of Australasia quality assurance panel for enterovirus detection by RT-PCR, and in the Quality Control for Molecular Diagnostics enterovirus typing panel.

Table 4: Enterovirus test results from samples originating in Australia, 1995 to 2022

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
2017[g]	1	1	204	92	173	471
2018[h]	2	0	231	89	198	520
2019[i]	1	0	52	97	97	247
2020[j]	1	0	91	135	20	247
2021	0	0	163	115	0	278
2022[k]	1	0	208	175	0	384

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 six 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.

g A Sabin-like poliovirus type 3 and a VDPV2 (non-Sabin-like) were isolated from sewage.

h Two separate isolations of Sabin-like poliovirus type 1 were identified from sewage.

i Sabin-like poliovirus type 3 was identified from sewage.

j Sabin-like poliovirus type 3 was identified from sewage.

k Sabin-like poliovirus type 3 was identified from sewage.

Discussion

In 2022, Australia recorded the highest rates ever reported for non-polio AFP cases and adequate stool collection, the two key WHO AFP surveillance performance indicators. These results underscore the strength of Australia's AFP surveillance system and come at a critical time when poliovirus was detected in a number of countries around the world that were previously thought to have interrupted poliovirus transmission.

In 2022, Australia reported a non-polio AFP rate of 1.69 cases per 100,000 children less than 15 years of age, meeting the WHO AFP surveillance target for the fifteenth year in a row. The notification of AFP cases via the APSU and the PAEDS systems has routinely met the international surveillance standard that assesses whether a country's AFP surveillance system is sensitive enough to detect an importation of wild poliovirus or cVDPV. Nevertheless, gaps in AFP surveillance were noted at the sub-national level with Queensland and Tasmania failing to meet the WHO surveillance target. This is in contrast to 2021, in which both states achieved the WHO surveillance target, while the Australian Capital Territory and South Australia failed to meet the surveillance target that year, which can be attributed to chance variation in jurisdictions with smaller populations.

Australia has never achieved the strict WHO surveillance target for adequate stool collection from 80% of non-polio AFP cases.[27] In 2020, the PAEDS network implemented an action plan to improve the rate of adequate stool collection from AFP cases, and this has been a significant factor in Australia reporting 63%, 62% and 72% of cases with adequate specimens in 2020, 2021 and 2022 respectively, the highest levels reported since AFP surveillance was established in 1995. Nevertheless, there is room for improvement, and stool collection rates and WHO AFP surveillance targets are discussed regularly at PAEDS and PEP meetings as part of an ongoing evaluation of barriers to collection and of opportunities for improvement. Based on an extended time frame of 60 days after the onset of paralysis, which WHO considers to be the maximum duration of poliovirus shedding, 84% of AFP cases in 2022 had two specimens collected within this extended time frame.[20]

Poliovirus was not detected in any of the specimens referred for AFP surveillance or enterovirus typing in 2022. The non-polio enteroviruses EV-A71 and EV-D68 are commonly regarded as enteroviruses of public health interest due to their association with neurological disease and outbreaks.[7,8] While there were three detections of EV-A71 and two of EV-D68 in 2022, the widespread distribution of these cases did not support a common transmission link for either virus. Although EV-D68 has been associated with acute flaccid myelitis, a distinct syndrome of AFP, it is more commonly associated with mild respiratory illness and indeed is more likely to be detected in respiratory specimens than faecal specimens.[28,29] Accordingly, the referral of respiratory specimens from AFP cases, in addition to stool specimens, would broaden enterovirus surveillance and increase the likelihood of detecting EV-D68, which has been discussed by the PEP.[28]

While 2021 saw only six cases of wild poliovirus reported worldwide, the lowest number ever recorded, in 2022, wild poliovirus was detected in two polio-free countries in Africa, which was a set-back for the Global Polio Eradication Initiative. While WPV1 continues to be detected in the two remaining endemic countries (Afghanistan and Pakistan), in 2022 both Malawi and Mozambique reported their first cases of WPV1 in at least 30 years, with WPV1 first detected in stool specimens collected in November 2021 from a five-year-old child from the Central Region of Malawi.[30,31] To date, there have been no further detections of WPV1 in Malawi; but in May 2022, a case of WPV1 was reported in Mozambique from a 12-year-old child from the Changara district, which borders Malawi and Zimbabwe.[9,31] Detection of another seven cases of WPV1 between April and August 2022 confirmed an outbreak of WPV1 in Mozambique. These detections mark

the first cases of wild poliovirus detected in Africa in more than six years, with nucleotide sequence analysis confirming the virus in both countries was genetically linked to a strain circulating in the Sindh province of Pakistan in 2019 and 2020.[12,31]

In addition to wild poliovirus, cVDPV continues to present a challenge for the Global Polio Eradication Initiative, particularly across the African region. Indeed, every year since 2017, more cases of polio have been caused globally by cVDPV than by wild poliovirus.[32] However, in 2022, the emergence of cVDPV2 in three high-income countries with high vaccine coverage at the national level has been significant. In both Jerusalem and London, routine environmental surveillance demonstrated the emergence of cVDPV2 isolates in wastewater samples collected between April and October 2022 (Jerusalem) and February and July 2022 (London), indicative of ongoing poliovirus transmission.[33,34] To date, no cases of cVDPV2 have been detected in London; but as of February 2023, one case of cVDPV2 linked to positive environmental samples has been reported in Israel.[35] Further, in July 2022, a case of poliomyelitis due to cVDPV2, in an unvaccinated adult from Rockland County, New York, marked the first case of poliomyelitis reported in the United States of America since 2013.[15,16] Related virus has subsequently been detected in 94 environmental samples collected across five counties in New York State between May 2022 and March 2023, as well as in two environmental samples collected in August 2022 from targeted sampling sites in Canada that have close connections to the communities in New York where the virus has been detected.[17,18]

Full genome sequencing of the cVDPV2 isolates detected in wastewater samples collected in Jerusalem, London and New York reveals that the viruses are genetically linked, with a number of unique mutations and a common genomic structure due to recombination with a non-polio enterovirus.[16,33,34] Notably, many of these shared genetic signatures are outside of the VP1 region that is typically used to characterise circulating polioviruses, highlighting the value of full genome sequencing.

While the WHO has long recognised the testing of environmental samples as a means of detecting the presence of wild poliovirus and VDPVs in polio-free countries, the recent use of wastewater surveillance to successfully track the spread of SARS-CoV-2 through communities during the COVID-19 pandemic, and the recent detections of cVDVP2 in environmental samples collected in developed countries, have highlighted the value of pathogen surveillance through wastewater samples to public health groups. Certainly within Australia there has been significant interest towards expanding the environmental surveillance program for poliovirus. In this regard, in 2022, the NERL established environmental surveillance in metropolitan Perth. While poliovirus was not detected in any of the samples tested, the isolation of non-polio enteroviruses, which are considered to be ubiquitous in environmental samples, in those samples collected from Perth demonstrates the feasibility of sending sewage samples across the country for testing at the NERL.

With increased interest in both environmental surveillance generally and specifically for poliovirus detection, Australia is well placed to expand its environmental surveillance activities. This would only serve to strengthen Australia's surveillance capabilities and add further support to Australia's polio-free status. The NERL is currently working to develop full genome sequencing and direct molecular detection methodologies to supplement environmental surveillance activities.

Acknowledgements

The authors thank the clinicians and healthcare workers who participated in the AFP surveillance program in 2022 as well as the teams at APSU and PAEDS. The active involvement of the laboratory members of the ERLNA is gratefully acknowledged. We thank Paige Alister for her valuable laboratory support in 2022. The poliovirus surveillance program co-ordinated by the NERL is funded by the Australian Government Department of Health and Aged Care, the Victorian Government Department of Health, and VIDRL.

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