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Australian National Enterovirus Reference Laboratory annual report, 2024

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Abstract

Having been declared polio-free by the World Health Organization (WHO) in 2000, Australia remains at risk of poliovirus importation until the virus is eradicated globally. Australia monitors for poliovirus by conducting surveillance for cases of acute flaccid paralysis (AFP) in children less than 15 years of age, as recommended by the 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 2024, no cases of poliomyelitis were reported from clinical surveillance and Australia reported 2.04 non-polio AFP cases per 100,000 children, thereby meeting the WHO's performance criterion for a sensitive surveillance system. Non-polio enteroviruses including enterovirus A71 were identified from clinical specimens collected from 14 AFP cases. Australia also performs enterovirus and wastewater surveillance to complement the clinical surveillance system focussed on children. In 2024, there were 21 different non-polio enterovirus types detected in 764 clinical specimens referred for enterovirus typing, while an ambiguous vaccine-derived poliovirus type 2 was detected through wastewater surveillance. In 2024, there were 99 cases of wild poliovirus reported from the two remaining endemic countries, Afghanistan and Pakistan. Another 319 cases of poliomyelitis due to circulating vaccine-derived poliovirus were reported across 21 countries.

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 officially declared polio-free.[4] Nevertheless, Australia, like all polio-free countries, remains at risk of poliovirus importation until the virus is eradicated globally. Australia has established clinical and virological surveillance systems to monitor its polio-free status, including clinical surveillance for cases of acute flaccid paralysis (AFP, a marker syndrome for poliomyelitis), as well as enterovirus and wastewater surveillance.

The clinical surveillance program follows the WHO recommendation of investigating 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 is reviewed by the Polio Expert Panel (PEP) and reported to the WHO as evidence of Australia's continued polio-free status.

Enterovirus and wastewater 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 monitor 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. Accordingly, the WHO recognises the testing of environmental samples, such as wastewater, as a means of detecting the presence of wild poliovirus and vaccine-derived poliovirus (VDPV) in polio-free countries.

Globally, only wild poliovirus type 1 (WPV1) continues to be detected in the two remaining endemic countries, Afghanistan and Pakistan, with the global eradication of wild poliovirus types 2 and 3 certified in 2015 and 2019 respectively.[9] In 2024, there were 99 cases of WPV1 reported, with 25 cases reported in Afghanistan and 74 cases reported in Pakistan.[10] This is a significant increase compared to the preceding three years when a total of 39 cases were reported.

Poliovirus outbreaks due to circulating VDPV (cVDPV) also continue to present a challenge for the global eradication program. cVDPV can emerge in areas with poor sanitation infrastructure in conjunction with sustained low polio vaccine coverage. Although the number of AFP cases related to cVDPV have continued to decline from a peak of 1,113 cases in 2020, more cases of paralysis are caused by VDPV than by wild poliovirus.[11] cVDPV was detected in 319 AFP cases and 277 environmental samples in 2024, with detections in 38 countries across the WHO African and Eastern Mediterranean Regions as well as in Finland, France, Germany, Indonesia, Poland, Spain and the United Kingdom.[12]

This report summarises the poliovirus surveillance program in Australia for 2024, 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, while acute flaccid paralysis is notifiable in Queensland.[13,14] 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,15] Additionally, 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 represents 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.[16] The faecal specimens are tested by virus culture at the NERL with funding from the Australian Government Department of Health, Disability and Ageing.

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:

- Poliomyelitis due to wild 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 the WHO for inclusion in the global AFP surveillance data published in the Weekly Epidemiological Record.[17] 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.[16] 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 2024 equated to 48 cases based on the Australian Bureau of Statistics estimate of Australia's population at 30 June 2023. The WHO surveillance performance indicator for specimen collection 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: +61 3 9342 9607; email: enterovirus@vidrl.org.au.

ii Questionnaire is available online at https://my.fuzee.com/apsu-vidrl/afpquestionnaire.html.

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).[18,19] Inoculated cell cultures are observed microscopically up to 14 days, for the presence of cytopathic effects that indicate likely infection with a poliovirus (L20B-positive cultures) or 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 12 WHO reverse transcription real-time polymerase chain reaction (RT-qPCR) assays with specific targets to determine whether the cultured isolate is a non-polio enterovirus, a wild poliovirus, an oral polio vaccine (OPV) strain, or a VDPV, using a process known as intratypic differentiation (ITD).[20] The NERL sequences the complete viral protein 1 (VP1) genomic region of all polioviruses. The genomic sequence of the VP1 region, which contains major neutralising antibody binding sites, 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.[21]

Wastewater surveillance

Wastewater surveillance was initially established by the NERL in regional New South Wales in 2010. Since October 2022, routine wastewater surveillance has included the testing of two monthly samples; the first alternating between the Eastern and Western treatment plants in Melbourne, and the second rotating between the Beenyup, Subiaco and Woodman Point treatment plants in Perth.

Up to April 2024, wastewater samples were processed according to the two-phase separation procedure published by the WHO.[22] In brief, 800 ml of wastewater was collected at the inlet to the wastewater treatment plant prior to any biological or chemical treatment. At the laboratory, 500 ml of the sample was vigorously shaken at 4 °C with dextran, polyethylene glycol and sodium chloride. The mixture was 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 was inoculated onto L20B and RD-A cell lines and observed microscopically for cytopathic effect as described in the 'Virus culture' section above.

After a period of parallel testing with the WHO two-phase separation method, from May 2024, wastewater samples were processed using an in-house size-exclusion and ultracentrifugation method. The new method involved clarifying 225 ml of wastewater using chloroform treatment and centrifugation, before passing through a 0.45 µm filter and concentrating by ultracentrifugation at 25,000 rpm for 2 hours. The concentrated pellet is re-suspended in a final volume of 2 ml, achieving a final concentration of 100×, with the concentrate inoculated onto L20B and RD-A cell lines as for virus culture.

Recently, several state jurisdictions implemented their own poliovirus wastewater surveillance programs independent of the NERL's wastewater surveillance activities funded by the Australian Government Department of Health, Disability and Ageing. From late 2022, the New South Wales State Government Department of Health (NSW Health) commenced poliovirus surveillance, with wastewater samples collected at four sewage treatment plants within Sydney and the Hunter Region (Bondi, Liverpool, Quakers Hill and Burwood Beach) tested for poliovirus and enterovirus RNA using in-house RT-qPCR assays. From July 2024, PathWest trialled their own poliovirus surveillance program with wastewater samples collected from the Beenyup, Subiaco and Woodman Point treatment plants using a direct detection next generation sequencing approach. In both cases, where a sample tests positive for poliovirus, the remaining wastewater sample is sent to the NERL for confirmatory testing using the methods described above.[23]

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, 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 RNA using a RT-qPCR assay directed to highly conserved genomic sequence in the 5' untranslated region (UTR).[24] 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 at least may be used to exclude the presence of poliovirus.

Results

Classification of AFP cases

In 2024, a total of 122 notifications of AFP cases were received (Table 1). Of these, 30 notifications were reported by the APSU surveillance system and 92 through PAEDS. Two notifications were deemed to be ineligible because the patient's age was 15 years or older. Twenty-two notifications were duplicates, notified by more than one source, whether by multiple clinicians through the APSU or by both the APSU and PAEDS systems.

The PEP classified 98 cases as non-polio AFP, a rate of 2.04 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 marks the seventeenth consecutive year in which Australia has achieved the WHO AFP surveillance target and denotes also the highest non-polio AFP rate ever reported in Australia.

Of the 98 non-polio AFP cases: fifteen cases were notified by both the APSU and PAEDS; 77 cases were notified through the PAEDS system only; and six cases were notified through the APSU system only. The six 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 acute disseminated encephalomyelitis were the most common causes of non-polio AFP in 2024, with the PEP classifying 34 and 12 cases respectively, with these two conditions. An additional eleven cases were classified as transverse myelitis, five cases as tick bite paralysis and three cases as botulism.

Notification of AFP cases by state and territory

In 2024, AFP cases were notified from all jurisdictions except the Northern Territory and Tasmania (Table 1). The non-polio AFP rate 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 all jurisdictions where cases were notified (Table 1).

Table 1: Notification of acute flaccid paralysis cases, 2024 by state and territory

Jurisdiction[a]	Estimated population aged < 15 years[b]	Expected number of AFP cases in 2024[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	83,150	1	1	0	0	1	1.00
NSW	1,495,063	15	28	2	0	26	1.73
NT	51,831	1	0	0	0	0	0.00
Qld	1,003,458	10	37	0	19	18	1.80
SA	312,481	3	5	0	2	3	1.00
Tas.	93,882	1	0	0	0	0	0.00
Vic.	1,206,052	12	44	0	1	43	3.58
WA	537,066	5	7	0	0	7	1.40
Australia	**4,782,983**	**48**	**122**	**2**	**22**	**98**	**2.04**

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 2023. Available at www.abs.gov.au.

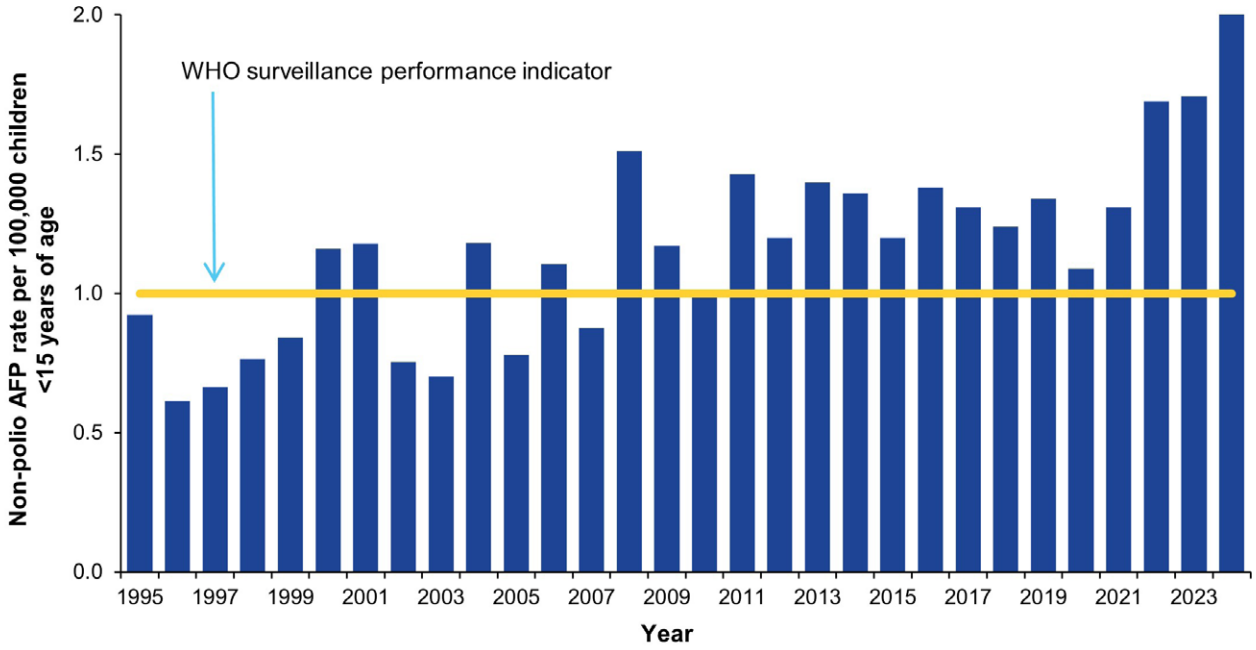
c The expected number of AFP cases for Australia is calculated by dividing the estimated population < 15 years of age by 100,000 and rounding to a whole number.

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

Table 2: Australia's surveillance for cases of acute flaccid paralysis, 2024, 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 2024)	98 cases classified as non-polio AFP	2.04 (98/48) 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)	57 AFP cases with adequate specimens collected	58% (57/98) classified non-polio AFP cases with adequate specimens

Figure 1: Non-polio acute flaccid paralysis rate, Australia 1995 to 2024[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 shown by the indicated horizontal line.

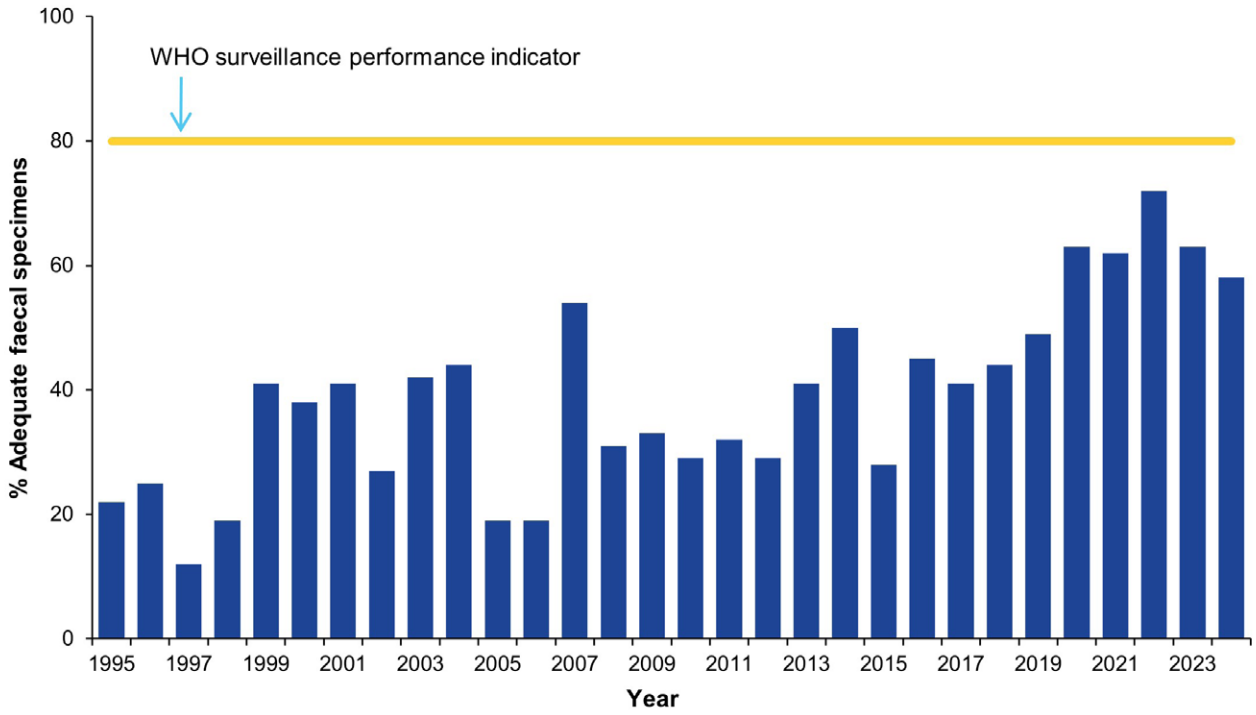
Faecal collection from AFP cases

In 2024, a total of 173 faecal specimens from 88 of the 98 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 57 of the eligible cases, satisfying the WHO criterion for adequate specimens and representing 58% of the non-polio AFP cases compared to the WHO benchmark of 80% (Figure 2, Table 2). While Australia has never attained the WHO adequate specimen benchmark, there has been a significant improvement in this measure since 2020, with the rate above 60% each year, compared to prior years in which the rate was frequently less than 50%. The result for 2024 marks the first time in five years the adequate specimen collection rate has fallen below 60% (Figure 2).

Enterovirus detections from AFP cases

Poliovirus was not detected in any of the specimens referred for AFP surveillance. Non-polio enteroviruses, including coxsackie A viruses, coxsackie B viruses, echoviruses and EV-A71, were identified from stool specimens collected from 14 separate AFP cases (Table 3). We note that enteroviruses are ubiquitous and a common childhood infection; identification of an enterovirus from a non-sterile site such as a faecal specimen does not infer causation, but may be an incidental finding.

Figure 2: Adequate faecal specimen collection rate, Australia 1995 to 2024[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 shown by the indicated horizontal line.

Table 3: Enteroviruses identified from Australian AFP cases, 2024

AFP case no.	State or territory[a]	Date of onset	Age at onset (years)	Enterovirus identified[b]	PEP diagnosis[c]
03	NSW	22-Jan-24	7.1	CVA4	ADEM
19	Qld	08-Apr-24	2.4	CVB2	GBS
20	Qld	17-Apr-24	4.9	E7	Viral myelitis
24	Vic.	18-Apr-24	1.4	CVB2	Bilateral Bell's' palsy
26	Vic.	26-Apr-24	2.9	species B	GBS
30	Qld	20-May-24	5.9	CVA19	GBS
31	NSW	08-May-24	13.9	E12	GBS
34	SA	24-May-24	0.2	EV-A71	Infantile botulism
48	NSW	23-Jun-24	3.7	E30	ADEM
57	WA	12-Jul-24	1.8	E25	GBS
73	NSW	31-Aug-24	2.8	CVB3	Enterovirus encephalomyelitis
74	Qld	29-Aug-24	3.3	E18	ADEM
85	Vic.	03-Oct-24	14.9	species A	TM
86	Vic.	03-Oct-24	3.8	EV-A71	Enterovirus encephalomyelitis

a NSW: New South Wales; Qld: Queensland; SA: South Australia; Vic.: Victoria; WA: Western Australia.

b CVA4: coxsackievirus A4; CVA19: coxsackievirus A19; CVB2: coxsackievirus B2; CVB3: coxsackievirus B3; E7: echovirus 7; E12: echovirus 12; E18: echovirus 18; E25: echovirus 25; E30: echovirus 30; EV-A71: enterovirus A71.

c ADEM: acute disseminated encephalomyelitis; GBS: Guillain-Barré syndrome; TM: transverse myelitis.

Wastewater surveillance

In 2024, the NERL tested 30 wastewater samples. Twenty-four samples were collected as part of a routine wastewater surveillance program that includes monthly collections in both Melbourne and Perth. Another six samples were referred from either Sydney Water in New South Wales or PathWest in Western Australia for confirmatory poliovirus testing following detection of poliovirus as part of those states' own wastewater surveillance programs.

Of the 24 samples collected for routine surveillance, a Sabin-like poliovirus type 1 and three Sabin-like poliovirus type 3 viruses were isolated from samples collected from the Eastern and Western treatment plants in Melbourne and from the Beenyup treatment plant in Perth (Table 4). In all cases, the VP1 nucleotide sequence had greater than 99% identity to the prototype Sabin vaccine strain, consistent with the polioviruses originating from recent vaccination events with OPV, perhaps in a returned traveller or visitor from a country that still uses OPV.

Of greater concern, a VDPV type 2 (VDPV2) virus was isolated from a wastewater sample collected from the Western treatment plant in Melbourne on 2 December (Table 4). Analysis of the VP1 nucleotide sequence revealed 124 nucleotide substitutions across the VP1 region compared to prototype Sabin 2 vaccine strain, equating to 13.7% divergence and immediately categorising the virus as a VDPV. Alignment of the VP1 sequence from this isolate with the VP1 sequence from an ambiguous VDPV2 (aVDPV2) isolate, previously detected in a wastewater sample collected from the Western treatment plant in November 2017, revealed 61 shared nucleotide substitutions and twelve shared amino acid substitutions compared to the prototype Sabin 2 vaccine strain. The 2024 isolate had an additional seven amino acids substitutions compared to the 2017 isolate. Additional sequence analysis by the Global Specialised Poliovirus Laboratory, at the United States Centers for Disease Control and Prevention, identified a number of sequence signatures typical of VDPVs from patients with primary immunodeficiencies. Based on these findings, the PEP and National Certification Committee for Poliomyelitis Eradication classified the virus as an aVDPV2 with genetic linkage to the aVDPV2 isolate from 2017, with the likely source of both viruses being an individual with primary immunodeficiency chronically infected with poliovirus.

Of the six samples referred for confirmatory testing, five samples were referred from Sydney Water; one collected from Liverpool and four collected from Quakers Hill treatment plants in Sydney. The NERL isolated Sabin-like poliovirus type 3 from all five samples (Table 4). The sixth sample was referred by PathWest for confirmatory testing following detection of Sabin-like poliovirus type 3 in a sample collected from the Woodman Point treatment plant in Perth on 23 October. Although the NERL did not isolate poliovirus by virus culture, detection of Sabin-like poliovirus type 3 was confirmed at the NERL by analysis of the complete VP1 sequence detected by PathWest, with the VP1 nucleotide sequence having 99.9% identity to the prototype Sabin 3 vaccine strain (Table 4).

In addition to the polioviruses detected, non-polio enteroviruses were isolated from all 30 wastewater samples tested, including the sample referred by PathWest for poliovirus confirmatory testing. Coxsackievirus B5, coxsackievirus B4 and echovirus 7 were the most common non-polio enteroviruses detected. Enterovirus infections are considered ubiquitous; the isolation of non-polio enteroviruses, from wastewater samples collected in polio-free countries not using OPV, serves as an indicator of the quality of the collection and test procedures in the absence of poliovirus detections.

Table 4: Polioviruses detected in wastewater samples collected in Australia, 2024

Collection site[a]	State or territory[b]	Date of collection	Poliovirus type[c]	No. of nucleotide changes from prototype[d]	% identity to prototype[d]
Western TP	Vic.	05-Feb-24	SL1	2	99.8
Quakers Hill TP	NSW	07-Feb-24	SL3	2	99.8
Eastern TP	Vic.	04-Mar-24	SL3	6	99.3
Quakers Hill TP	NSW	08-Mar-24	SL3	0	100.0
Liverpool TP	NSW	13-Mar-24	SL3	2	99.8
Western TP	Vic.	03-Jun-24	SL3	0	100.0
Beenyup TP	WA	09-Sep-24	SL3	2	99.8
Woodman Point TP	WA	23-Oct-24	SL3	1	99.9
Quakers Hill TP	NSW	15-Nov-24	SL3	0	100.0
Quakers Hill TP	NSW	21-Nov-24	SL3	0	100.0
Western TP	Vic.	02-Dec-24	VDPV2	124	86.3

a TP: treatment plant.

b NSW: New South Wales; Vic.: Victoria; WA: Western Australia.

c SL1: Sabin-like poliovirus type 1; SL3: Sabin-like poliovirus type 3; VDPV2: vaccine-derived poliovirus type 2.

d Number of nucleotide (nt) changes and % identity across the poliovirus (PV) VP1 region, which is 906nt for PV1, 903nt for PV2 and 900nt for PV3.

Table 5: Laboratory results for specimens and wastewater samples collected in Australia, 2024

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
Vaccine-derived poliovirus type 2	0	0	1	0	1
Sabin poliovirus type 1	0	0	1	0	1
Sabin poliovirus type 3	0	0	9	0	9
Rhinovirus	0	0	0	6	6
Non-polio enterovirus	23	1	30	564	618
No enterovirus identified	153	3	0	76	232
Total	**176**	**4**	**41**	**646**	**867**

a A total of 30 wastewater samples were tested, with both poliovirus and a non-polio enterovirus detected in eleven samples.

b A total of 764 specimens were referred for enterovirus typing, with 118 specimens being inadequate for testing.

Enterovirus surveillance

In 2024, a total of 764 clinical specimens were referred to the NERL for enterovirus typing (Table 5). The majority of specimens (68.6%) were referred from South Australia, followed by Victoria (23.9%), with the remaining specimens (7.4%) referred from the Australian Capital Territory, New South Wales, the Northern Territory, Queensland and Tasmania. Nearly 90% of referred specimens were collected from children aged less than 15 years, with 169 specimens (22.3%) collected from neonates (0 to < 3 months), 111 specimens (14.6%) collected from post-neonatal infants (3 months to < 1 year) and 349 specimens (46.0%) collected from young children (1 to < 5 years). Blood was the most common specimen type received (55.9%), followed by mouth, skin and vesicle swabs (20.5%); cerebrospinal fluid (12.2%); and respiratory specimens (5.1%).

Of the specimens received for enterovirus typing, 646 (84.6%) were suitable for testing. Of these specimens, 553 (85.6%) were characterised as non-polio enteroviruses, with 371 (57.4%) being fully typed based on VP1 sequence and 182 (28.2%) characterised only as *Enterovirus* species. Of the remaining specimens, 76 (11.8%) were reported as no enterovirus identified, eleven (1.7%) as mixed enteroviruses that could not be resolved by sequencing and six (0.9%) characterised as *Rhinovirus* (a species of the *Enterovirus* genus) (Table 5).

Poliovirus was not detected in any of the specimens referred for enterovirus typing. A total of 21 different enterovirus types were reported: the most common detections were coxsackievirus A6 (25.1%), associated with hand foot and mouth disease, followed by coxsackievirus B3 (8.3%) and echovirus 7 (4.9%).

In 2024, including specimens received for AFP and environmental surveillance, a total of 400 non-polio enteroviruses were typed and an additional 218 enteroviruses were characterised to the level of *Enterovirus* species by the NERL (Table 5). Excluding rhinoviruses, a total of 861 enterovirus typing results were reviewed by the NERL, with no additional typing results referred from members of the ERLNA (Table 6).

Polio regional reference laboratory activities

In 2024, as part of its role as a Polio Regional Reference Laboratory, the NERL received a total of 145 stool specimens from AFP cases referred from Brunei Darussalam (three cases), Pacific Island countries (seventeen cases) and Papua New Guinea (54 cases). Sabin-like poliovirus type 1 together with Sabin-like poliovirus type 3 was identified in one case from Papua New Guinea. The VP1 nucleotide sequence of both the Sabin 1 and Sabin 3 isolates shared at least 99.6% identity to the prototype Sabin vaccine strains, indicative of a recent vaccination with OPV. Non-polio enteroviruses were reported from four AFP cases from the Pacific Islands and 24 cases from Papua New Guinea, with numerous species C enteroviruses detected including coxsackievirus A13, A19, A22 and A24, and enterovirus C96 and C99.

Quality assurance programs

In 2024, the NERL 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 6: Enterovirus test results from samples originating in Australia, 1995 to 2024

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
2023[l]	3	0	644	277	0	924
2024[m]	10	1	618	232	0	861

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.

l Three separate isolations of Sabin-like poliovirus type 3 were identified from wastewater.

m Eleven separate isolations of poliovirus were identified from wastewater including a VDPV2 (non-Sabin-like).

Discussion

With increasing detections of wild poliovirus in both AFP cases and environmental samples in Afghanistan and Pakistan, and ongoing circulation of VDPV in many regions across the globe, 2024 was a challenging year for the Global Polio Eradication Initiative. Closer to Australia, outbreaks of cVDPV have occurred in Papua New Guinea in 2018, concurrently in Malaysia and the Philippines between 2019 and 2020, and more recently in Indonesia, with eight cases of poliomyelitis due to cVDPV2 reported in 2024.[25-27] With ongoing circulation of wild poliovirus and VDPV, Australia, like all polio-free countries, remains at risk of poliovirus importation, highlighting the crucial need to maintain high vaccination rates and sensitive polio surveillance systems.

In 2024, Australia reported a non-polio AFP rate of 2.04 cases per 100,000 children less than 15 years of age, the highest non-polio AFP rate recorded in Australia and the seventeenth year in a row in which Australia has achieved the WHO non-polio AFP surveillance target. This result underscores the strength of Australia's AFP surveillance system. At the sub-national level, the Northern Territory and Tasmania were the only jurisdictions failing to meet the WHO non-polio AFP surveillance target. This is likely due to both jurisdictions having fewer than 100,000 children under 15 years of age; the non-polio AFP rate is monitored long-term for under-reporting. 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. Although Australia has never achieved the strict WHO surveillance target for adequate stool collection from 80% of non-polio AFP cases, 77% of cases in 2024 had at least one specimen collected within 14 days of the onset of paralysis and tested to exclude poliovirus as the causative agent.

Enterovirus and wastewater surveillance supplement Australia's AFP surveillance program, providing additional means of monitoring Australia's polio-free status. In 2024, poliovirus was not detected in any of the 826 specimens referred for AFP surveillance or enterovirus typing, but ten Sabin-like polioviruses and a type 2 VDPV were isolated from wastewater samples collected in Melbourne, Perth and Sydney. Genetic sequencing confirmed each of the Sabin-like polioviruses had at least 99% identity to the prototype Sabin vaccine strain, indicating the likely source to have been a visitor or returned traveller from a country that still uses OPV, since Australia replaced this vaccine with inactivated polio vaccine in 2005.

While the NERL has been performing wastewater surveillance in some capacity since 2010 as recommended by WHO, following the COVID-19 pandemic there has been increased interest towards expanding the environmental surveillance program for poliovirus. In this regard, in 2022, the NERL established wastewater surveillance in metropolitan Perth, in addition to the monthly collections in Melbourne. Then, in late 2022, NSW Health commenced its own wastewater surveillance program at a number of sites within Sydney and the Hunter Region, and in 2024 PathWest also established wastewater testing. Alternative testing strategies utilised by other groups have often presented a challenge to confirm the reported poliovirus detections by virus culture, leading VIDRL to develop an alternative wastewater concentration method that has shown increased sensitivity for isolation of polioviruses in culture versus the WHO method. The NERL is also continuing to develop direct detection methodologies and full genome sequencing to supplement existing wastewater surveillance capabilities.

Ongoing replication of an OPV strain can lead to the emergence of a VDPV that has a significant number of nucleotide changes compared to prototype vaccine strain and has regained the neurovirulence and transmission characteristics of wild poliovirus. Where prolonged replication occurs in a single host with a primary immunodeficiency, the emergent VDPV is referred to as an immunodeficiency-related VDPV (iVDPV). Where prolonged replication occurs through serial transmission in an under-vaccinated community, the emergent VDPV is referred to as a cVDPV. The VDPV2 virus isolated from a wastewater sample collected from the Western treatment plant in Melbourne on 2 December had 124 nucleotide substitutions across the VP1 region compared to the prototype Sabin 2 vaccine strain, and was genetically linked to a VDPV2 virus isolated from wastewater collected from the same treatment plant in Melbourne in December 2017. Although both isolates have been classified as ambiguous VDPV as their ultimate source is unknown, sequence analysis indicates the likely source of the isolates is the same individual with a primary immunodeficiency.

The WHO recognises iVDPVs to be of interest to the polio eradication program due to the risk of long-term shedding of potentially neurovirulent polioviruses and has established guidelines for implementing poliovirus surveillance among patients with primary immunodeficiency disorders (PID) related to a lack of B cells, who are at risk of a chronic poliovirus infection if immunised or infected with the live poliovirus strains present in OPV. The PEP and National Certification Committee for Poliomyelitis Eradication supported the NERL undertaking a pilot study to screen patients with PID for poliovirus infection and an ethics application is currently being reviewed at a major tertiary hospital for this purpose.

With increased interest in wastewater surveillance for poliovirus detection and a pilot study to screen patients with PID, Australia is well placed to further expand its poliovirus surveillance activities. This will serve to strengthen Australia's surveillance capabilities to monitor Australia's polio-free status.

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