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Australian National Enterovirus Reference Laboratory annual report, 2023

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Annual report

Australian National Enterovirus Reference Laboratory annual report, 2023

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 2023, no cases of poliomyelitis were reported from clinical surveillance and Australia reported 1.71 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 A9, coxsackievirus B5, echovirus 9, echovirus 30, enterovirus A71 and enterovirus C96 were identified from clinical specimens collected from AFP cases. Australia also performs enterovirus and wastewater surveillance to complement the clinical system focussed on children. In 2023, there were twelve cases of wild poliovirus reported from the last two remaining endemic countries: Afghanistan and Pakistan. Another 23 countries 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 established clinical and virological surveillance systems to monitor its polio-free status in 1995. 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 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. The WHO recognises the testing of environmental samples, such as wastewater and river water, 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 2023, only twelve cases of WPV1 were reported, with six cases reported each in Afghanistan and Pakistan.[10] Since 2020, both countries have continued to make progress towards the eradication of wild poliovirus, as demonstrated by declining numbers of poliomyelitis cases and positive environmental samples, geographically localised transmission and a reduction in the number of transmission chains to a single active chain in each country.[10,11] It is anticipated the world is on the cusp of permanently interrupting wild poliovirus transmission.

Nevertheless, polio outbreaks due to circulating VDPV (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 decreased in 2023 compared to 2022, cVDPV continues to present a challenge for the global polio eradication program.[12] cVDPV was detected in 520 AFP cases and 418 environmental samples in 2023, with detections across 21 countries in the WHO African and Eastern Mediterranean Regions as well as Indonesia and Israel.[12]

This report summarises the poliovirus surveillance program in Australia for 2023, 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 available online.[15,ii] 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, but represent 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 and Aged Care.

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

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

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 2023 equated to 48 cases based on the Australian Bureau of Statistics estimate of Australia's population at 30 June 2022. 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.

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 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 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 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.[21]

Environmental surveillance

Environmental surveillance was initially established by the NERL in regional New South Wales in 2010. Since 2014, testing has focussed on metropolitan Melbourne with wastewater samples collected from both the Eastern and Western Treatment Plants. In 2022, environmental surveillance was expanded to include testing of wastewater 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.[22] In brief, 800 ml of wastewater 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.

In 2022, the New South Wales Government Department of Health (NSW Health) commenced its own poliovirus wastewater surveillance program. The program tests untreated composite wastewater samples collected at four sewage treatment plants within Sydney and the Hunter Region (Bondi, Liverpool, Quakers Hill and Burwood Beach). Wastewater samples are collected by Sydney Water and tested for Sabin poliovirus types 1, 2 and 3, wild poliovirus and enterovirus RNA using an in-house RT-qPCR assay. Where a sample tests positive for poliovirus, 500 ml of the sample is sent to the NERL for confirmatory testing using the WHO protocol described above.

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).[23] 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 2023, a total of 94 notifications of AFP cases were received (Table 1). Of these, 15 notifications were reported by the APSU surveillance system and 79 through PAEDS. Four notifications were deemed to be ineligible: one because the patient's age was 15 years or older; and three because the clinical presentation was subsequently determined not to be AFP.

The PEP classified 82 cases as non-polio AFP, a rate of 1.71 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 sixteenth consecutive year in which Australia has achieved the WHO AFP surveillance target and the highest non-polio AFP rate ever reported in Australia.

Of the 82 non-polio AFP cases: eight cases were notified by both the APSU and PAEDS systems; 70 cases were notified through the PAEDS system only; and four cases were notified through the APSU system only. The four 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 2023, with the PEP classifying 26 and 15 cases respectively with these two conditions. Thirteen cases were classified as acute disseminated encephalomyelitis and three as myasthenia gravis.

Table 1: Notification of acute flaccid paralysis cases, 2023 by state and territory

State or territory[a]	Estimated population aged < 15 years[b]	Expected number of AFP cases in 2023[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	83,328	1	1	0	0	1	1.00
NSW	1,493,112	15	20	0	1	19	1.27
NT	52,578	1	4	1	0	3	3.00
Qld	999,525	10	16	1	7	8	0.80
SA	311,902	3	3	0	0	3	1.00
Tas.	95,209	1	2	0	0	2	2.00
Vic.	1,191,384	12	41	1	0	40	3.33
WA	531,587	5	7	1	0	6	1.20
Australia	**4,758,625**	**48**	**94**	**4**	**8**	**82**	**1.71**

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 2022. Available at www.abs.gov.au.

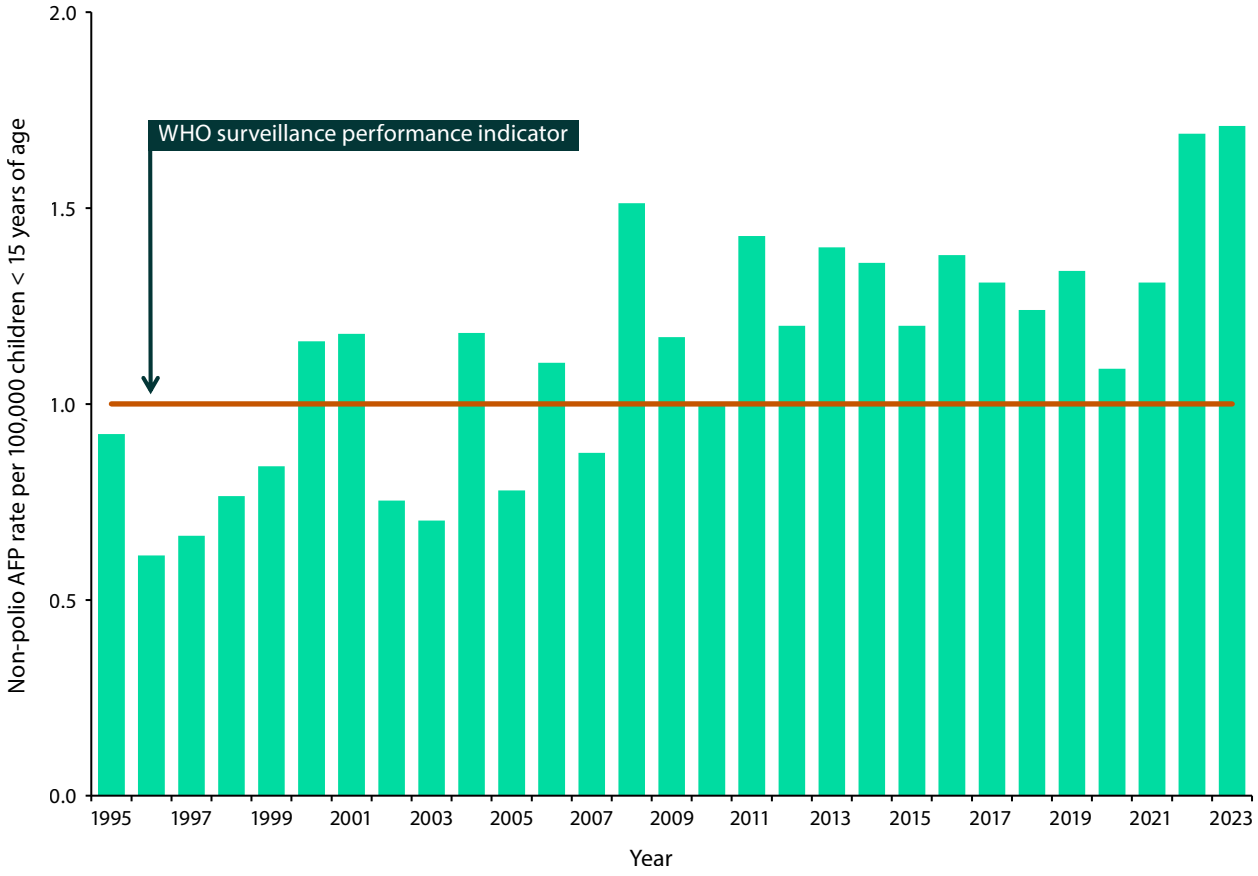
c The expected number of AFP cases for each jurisdiction is calculated by dividing the estimated population < 15 years of age by 100,000 and rounding to a whole number.

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, 2023, 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 2023)	82 cases classified as non-polio AFP	1.71 (82 / 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)	52 AFP cases with adequate specimens collected	63% (52 / 82) classified non-polio AFP cases with adequate specimens

Figure 1: Non-polio acute flaccid paralysis rate, Australia 1995 to 2023[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 orange line.

Notification of AFP cases by state and territory

In 2023, AFP cases were notified from all jurisdictions in Australia (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 all jurisdictions except Queensland.

Faecal collection from AFP cases

In 2023, a total of 150 faecal specimens from 77 of the 82 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 52 of the eligible cases, satisfying the WHO criterion for adequate specimens and representing 63% 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 each year from 2020 onwards marks a significant improvement from previous years in which the proportion of adequate stools was frequently less than 50%; the observed trend demonstrates a continuing improvement in this performance metric (Figure 2).

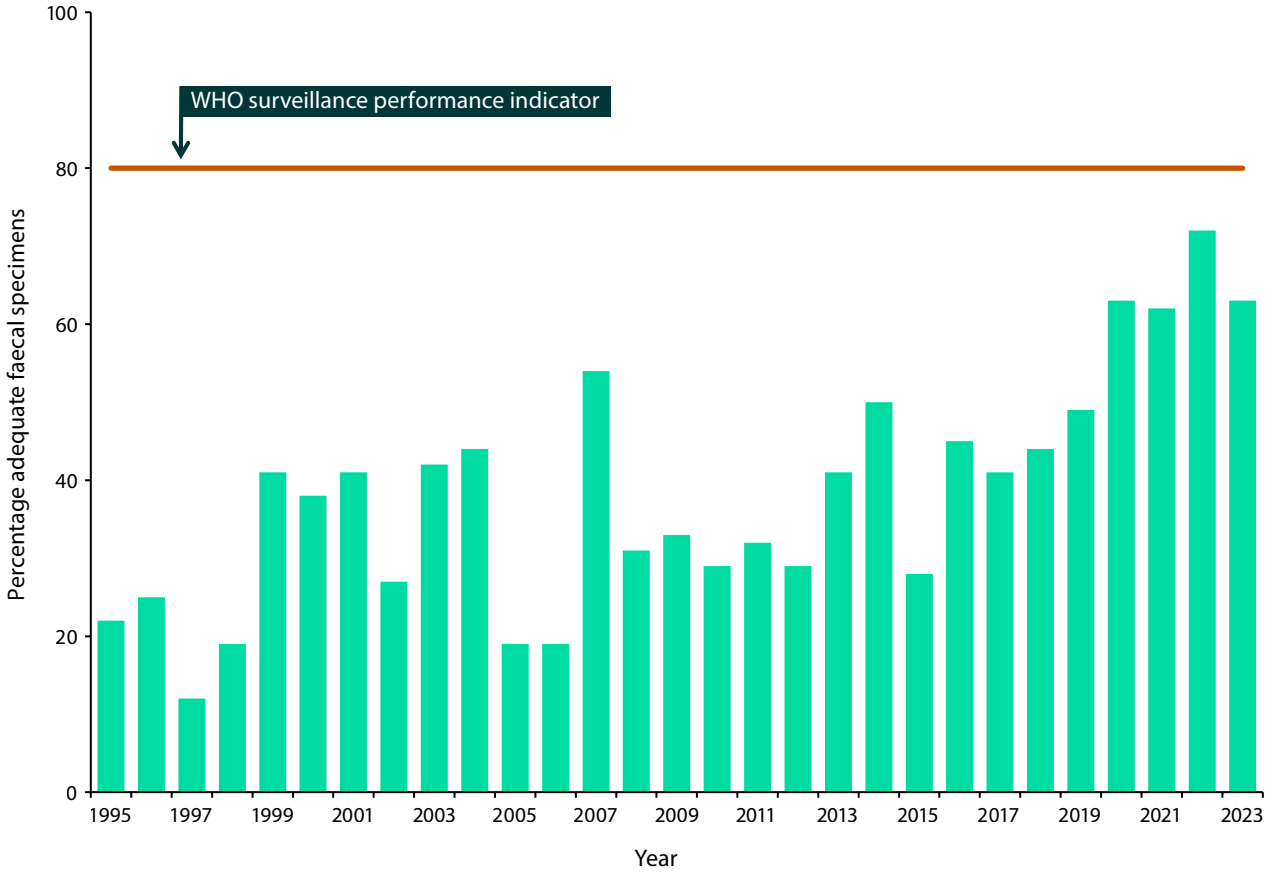
Poliovirus was not detected in any of the specimens referred for AFP surveillance. Non-polio enteroviruses, including coxsackievirus A9, coxsackievirus B5, echovirus 9, echovirus 30, EV-A71 and enterovirus C96 were identified from stool specimens collected from seven separate AFP cases: one in New South Wales (echovirus 30), two in the Northern Territory (coxsackievirus B5, enterovirus C96), and four in Victoria (coxsackievirus A9, coxsackievirus B5, echovirus 9, EV-A71). Non-polio enteroviruses that could only be characterised as *Enterovirus* species due to low viral load were identified from stool specimens collected from another two AFP cases: one in South Australia (*Enterovirus B*) and one in Victoria (*Enterovirus A*).

Wastewater surveillance

In 2023, the NERL tested 42 wastewater samples. Twenty-four of the samples were collected as part of a routine wastewater surveillance programme, which includes monthly sample collections alternating between the Eastern and Western Treatment Plants in Melbourne, and a second monthly sample collection rotating between Beenyup, Subiaco and Woodman Point Treatment Plants in Perth. The remaining 18 samples were referred from Sydney Water for poliovirus testing.

Of the 24 samples collected for routine surveillance, Sabin-like poliovirus type 3 was isolated from a sample collected from the Western Treatment Plant in Melbourne on 2 October and from a sample collected from the Woodman Point Treatment Plant in Perth on 13 November. In both cases, the nucleotide sequence of the VP1 region shared at least 99.8% identity with the prototype Sabin vaccine strain, consistent with the poliovirus originating from a recent vaccination event with OPV, perhaps in a returned traveller or visitor from a country that still uses OPV.

Figure 2: Adequate faecal specimen collection rate, Australia 1995 to 2023[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 orange line.

Of the 18 samples referred from Sydney Water, 17 samples were collected from the Quakers Hill Treatment Plant in New South Wales between 4 September and 9 December 2023. Neither Sydney Water nor the NERL detected poliovirus in any of these samples. The remaining wastewater sample was referred by Sydney Water following detection of poliovirus in a sample collected from the Liverpool Wastewater Treatment Plant in NSW on 23 September. The NERL isolated a Sabin-like poliovirus type 3 from this sample and the nucleotide sequence of the VP1 region was 100% identical to the prototype vaccine strain, indicative of a recent vaccination event with OPV in another country.

Non-polio enteroviruses were isolated from all 42 wastewater samples tested, with coxsackievirus B5 the most common enterovirus detected, identified in 60% (25/42) of samples. Other non-polio enteroviruses detected included coxsackievirus A4, coxsackievirus B3, coxsackievirus B4 and echovirus 9. Enterovirus infections are considered ubiquitous and 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.

Enterovirus surveillance

In 2023, a total of 750 clinical specimens were referred to the NERL for enterovirus typing (Table 3). The majority of specimens (68.0%) were referred from South Australia, followed by Victoria (27.1%), with the remaining specimens (4.9%) referred from the Australian Capital Territory, the Northern Territory, Tasmania and Western Australia. Of these specimens, 588 (78.4%) were characterised as non-polio enteroviruses, with 341 (58.0%) being fully typed based on VP1 sequence and 247 (42.0%) characterised only as *Enterovirus* species. Of the remaining specimens, eight (1.1%) were characterised as *Human rhinovirus* (a species of the *Enterovirus* genus), 137 (18.2%) were reported as no enterovirus identified and 17 (2.3%) were inadequate for testing (Table 3). Poliovirus was not detected in any of the specimens referred for enterovirus typing.

In 2023, including specimens received for AFP and environmental surveillance, a total of 380 non-polio enteroviruses were typed and an additional 264 enteroviruses were characterised to the level of *Enterovirus* species by the NERL (Table 3). Excluding rhinoviruses, a total of 924 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 2023 were coxsackievirus A6, coxsackievirus A10, coxsackievirus B5 and coxsackievirus B1, which together accounted for 73% (279/380) of all enteroviruses typed in 2023.

Table 3: Laboratory results for specimens and wastewater samples collected in Australia, 2023

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	3	0	3
Rhinovirus	0	0	0	8	8
Non-polio enterovirus	14	0	42	588	644
No enterovirus identified	133	7	0	137	277
Total	**147**	**7**	**45**	**733**	**932**

a A total of 42 wastewater samples were tested, with both Sabin poliovirus type 3 and a non-polio enterovirus detected in three samples.

b A total of 750 specimens were referred for enterovirus typing, with 17 specimens being inadequate for testing.

Table 4: Enterovirus test results from samples originating in Australia, 1995 to 2023

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

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.

Polio regional reference laboratory activities

In 2023, as part of its role as a Polio Regional Reference Laboratory, the NERL received four stool specimens from two AFP cases in Brunei Darussalam; 35 stool specimens from 18 AFP cases in Fiji; eight stool specimens from five AFP cases in Solomon Islands; and three stool specimens from one AFP case in Tonga. Non-polio enteroviruses including, coxsackievirus A13, echovirus 14 and enterovirus C96 were identified from stool specimens collected from four separate AFP cases: two from Fiji (echovirus 14, enterovirus C96) and two from Solomon Islands (coxsackievirus A13, echovirus 14). Non-polio enteroviruses that could only be characterised as *Enterovirus* species, due to low viral load, were identified from stool specimens collected from another four AFP cases: three from Fiji (two *Enterovirus B*, one *Enterovirus C*) and one from Tonga (*Enterovirus C*). Poliovirus was not isolated from any of the specimens.

A total of 138 stool specimens were received from Papua New Guinea and tested by the NERL, including 126 specimens from 67 AFP cases involving children less than 15 years of age, seven specimens from four AFP cases involving patients 15 years of age or greater, and one specimen each from five contacts of AFP cases. Sabin-like poliovirus type 1 together with Sabin-like-poliovirus type 3 was identified in five specimens from three AFP cases; Sabin-like poliovirus type 3 alone was identified in a further seven specimens from four AFP cases. In all cases, the nucleotide sequence of the poliovirus VP1 region had at least 99.6% identity with the prototype vaccine strain, indicative of recent vaccination with OPV. Non-polio enteroviruses were detected in 40.5% (56/138) of the specimens, with several species C enteroviruses detected, including coxsackievirus A13, coxsackievirus A21, coxsackievirus A24 and enterovirus C99.

Quality assurance programs

In 2023, the NERL maintained its accreditation as a WHO Polio Regional Reference Laboratory through successful completion of the WHO quality assurance panel for poliovirus isolation. The NERL also successfully participated in the Royal College of Pathologists of Australasia quality assurance panel for enterovirus detection by RT-PCR; and the Quality Control for Molecular Diagnostics enterovirus typing panel.

Discussion

Although only 12 cases of wild poliovirus were reported from the last remaining endemic regions of Afghanistan and Pakistan in 2023, cVDPV continues to present a challenge for the Global Polio Eradication Initiative.[10] Indeed, every year since 2017, more cases of polio have been caused globally by cVDPV than by wild poliovirus.[24] Within our region, 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 six cases of poliomyelitis related to cVDPV type 2 reported between October 2022 and November 2023.[25–27] Ongoing circulation of wild poliovirus and cVDPV is a risk to people everywhere and highlights the crucial need to maintain high levels of polio vaccine coverage and sensitive polio surveillance systems.

In 2023, Australia reported a non-polio AFP rate of 1.71 cases per 100,000 children less than 15 years of age, the highest non-polio AFP rate ever recorded in Australia and the sixteenth year in a row in which Australia has achieved the WHO AFP surveillance target. This result underscores the strength of Australia's AFP surveillance system. At the subnational level, Queensland was the only jurisdiction failing to meet the WHO surveillance target. 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.

Australia has never achieved the strict WHO surveillance target for adequate stool collection from 80% of non-polio AFP cases.[28] 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 higher proportions of cases with adequate specimens over the past four years. 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 opportunities for improvement. Based on an extended time frame of 60 days after the onset of paralysis, which WHO has set as the maximum for specimen collection, 79% of AFP cases in 2023 had two specimens collected within this period.[16]

Enterovirus and wastewater surveillance supplement Australia's AFP surveillance program, providing additional means of monitoring Australia's polio-free status. In 2023, poliovirus was not detected in any of the 887 specimens referred for AFP surveillance or enterovirus typing, but was isolated from three separate wastewater samples, one each collected in Melbourne, Perth and Sydney. Genetic sequencing determined each of these isolates to be a Sabin-like poliovirus type 3 strain, with at least 99.8% 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.

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 use of wastewater surveillance to successfully track the spread of SARS-CoV-2 through communities during the COVID-19 pandemic and recent detections of cVDVP in environmental samples collected in developed countries has 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 wastewater surveillance in metropolitan Perth, in addition to the existing monthly collections in Melbourne, and NSW Health commenced its own poliovirus wastewater surveillance program at a number of sites within Sydney and the Hunter Region.

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

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References

1. Aylward RB. Poliomyelitis. In Heymann DL, ed. *Control of Communicable Diseases Manual*. 20th ed. Fort Worth: APHA Press, 2015;477–84. doi: https://doi.org/10.2105/CCDM.2745.116.

2. Nathanson N, Kew OM. From emergence to eradication: the epidemiology of poliomyelitis deconstructed. *Am J Epidemiol*. 2010;172(11):1213–29. doi: https://doi.org/10.1093/aje/kwq320.

3. World Health Organization (WHO). Poliomyelitis. [Internet.] Geneva: WHO; 24 October 2023. [Accessed on 26 March 2024.] Available from: https://www.who.int/news-room/fact-sheets/detail/poliomyelitis.

4. Adams T. Farewell to polio in the Western Pacific. *Bull World Health Organ*. 2000;78(12):1375.

5. Australian Paediatric Surveillance Unit (APSU). *Study Protocol: Acute Flaccid Paralysis*. [Internet.] Sydney: APSU; 2024. [Accessed on 26 March 2024.] Available from: https://www.apsu.org.au/assets/current-studies/AFP-Study-Protocol-June-2.pdf.

6. Paediatric Active Enhanced Disease Surveillance (PAEDS). Surveillance and research: acute flaccid paralysis. [Internet.] Sydney: National Centre for Immunisation Research and Surveillance, PAEDS; 2022. [Accessed on 26 April 2024.] Available from: http://www.paeds.org.au/our-work/surveillance-and-research.

7. Puenpa J, Wanlapakorn N, Vongpunsawad S, Poovorawan Y. The history of enterovirus A71 outbreaks and molecular epidemiology in the Asia-Pacific region. *J Biomed Sci*. 2019;26(1):75. doi: https://doi.org/10.1186/s12929-019-0573-2.

8. Sun J, Hu XY, Yu XF. Current understanding of human enterovirus D68. *Viruses*. 2019;11(6):490. doi: https://doi.org/10.3390/v11060490.

9. WHO. Polio and Prevention. The Virus. [Internet.] Geneva: WHO; 7 October 2016. [Accessed: 26 March 2024.] Available from: https://polioeradication.org/polio-today/polio-prevention/the-virus/.

10. WHO. *Global wild poliovirus 2018-2024*. Geneva: WHO; 26 March 2024. [Accessed on 26 March 2024.] Available from: https://polioeradication.org/wp-content/uploads/2024/03/weekly-polio-analyses-WPV-20240326.pdf.

11. WHO. Statement of the thirty-fifth Polio IHR Emergency Committee. [Internet.] Geneva: WHO; 12 May 2023. [Accessed on 26 March 2024.] Available from: https://www.who.int/news/item/12-05-2023-statement-of-the-thirty-fifth-polio-ihr-emergency-committee.

12. WHO. Global circulating vaccine-derived poliovirus. [Internet.] Geneva: WHO; 26 March 2024. [Accessed on 26 March 2024.] Available from: https://polioeradication.org/this-week/variant-polio-cvdpv-cases/.

13. Australian Government Department of Health and Aged Care. Poliovirus infection. [Internet.] Canberra: Australian Government Department of Health and Aged Care; 12 July 2022. [Accessed on 26 March 2024.] Available from: http://www.health.gov.au/diseases/poliovirus-infection.

14. Queensland Government Department of Health (Queensland Health). Acute flaccid paralysis (AFP). [Internet.] Brisbane: Queensland Health; 20 July 2022. [Accessed on 26 March 2024.] Available from: https://www.health.qld.gov.au/disease-control/conditions/acute-flaccid-paralysis-afp.

15. APSU. Current Studies. Acute Flaccid Paralysis (AFP). [Internet.] Sydney: APSU; 2024. [Accessed on 26 March 2024.] Available from: https://apsu.org.au/studies/current/#acute-flaccid-paralysis.

16. WHO. *Polio: Vaccine Preventable Diseases Surveillance Standards*. Geneva: WHO; 4 September 2018. [Accessed on 26 March 2024] Available from: https://www.who.int/publications/m/item/vaccine-preventable-diseases-surveillance-standards-polio.

17. WHO. *Weekly epidemiological record (WER)*. Geneva: WHO; 22 March 2024. [Accessed on 26 March 2024] Available from: https://iris.who.int/handle/10665/376320.

18. Wood DJ, Hull B. L20B cells simplify culture of polioviruses from clinical samples. *J Med Virol*. 1999;58(2);188–92. doi: https://doi.org/10.1002/(SICI)1096-9071(199906)58:2<188::AID-JMV15>3.0.CO;2-H.

19. WHO. *Polio Laboratory Manual*, 4th edition. (WHO/IVB/04.10) Geneva: WHO, Department of Immunization, Vaccines and Biologicals; 2004.

20. Sun H, Harrington C, Gerloff N, Mandelbaum M, Jeffries-Miles S, Apostol LNG et al Validation of a redesigned pan-poliovirus assay and real-time PCR platforms for the global poliovirus laboratory network. *PloS One*. 2021;16(8):e0255795. doi: https://doi.org/10.1371/journal.pone.0255795.

21. Stewardson AJ, Roberts JA, Beckett CL, Prime HT, Loh PS, Thorley BR et al. Imported case of poliomyelitis, Melbourne, Australia, 2007. *Emerg Infect Dis*. 2009;15(1):63–5. doi: https://doi.org/10.3201/eid1501.080791.

22. WHO. *Guidelines for environmental surveillance of poliovirus circulation*. Geneva: WHO, Department of Vaccines and Biologicals; 2003. Available from: https://iris.who.int/handle/10665/67854.

23. Roberts JA. Thesis. "Chapter 2: Development of a Novel Enterovirus Detection and Super-Speciation Assay", An integrated bioinformatics and computational biophysics approach to enterovirus surveillance and research. RMIT University, 2014: 62-109. [Accessed on 26 April 2024.] Available from: https://researchbank.rmit.edu.au/view/rmit:162129.

24. Venkatesan P. Global polio eradication set back by COVID-19 pandemic. *Lancet Microbe*. 2022:3(3):e172. doi: https://doi.org/10.1016/S2666-5247(22)00042-8.

25. Raphael M, Adam DC, Chughtai AA, Kelly-Hanku A, Hutchinson D, Lai YA et al. Poliomyelitis outbreak in Papua New Guinea, 2018. *Global Biosecurity*. 2022;4(1). doi: https://doi.org/10.31646/gbio.164.

26. Snider CJ, Boualam L, Tallis G, Takashima Y, Abeyasinghe R, Lo YR et al. Concurrent outbreaks of circulating vaccine-derived poliovirus types 1 and 2 affecting the Republic of the Philippines and Malaysia, 2019–2021. *Vaccine*. 2023;41(Suppl 1):A58–69. doi: https:doi.org/10.1016/j.vaccine.2022.02.022.

27. WHO. Disease Outbreak News: Circulating vaccine-derived poliovirus type 2 (cVDPV2) – Indonesia. [Internet.] Geneva: WHO; 11 January 2024. [Accessed on 26 March 2024.] Available from: https://www.who.int/emergencies/disease-outbreak-news/item/2024-DON500.

28. May M, Durrheim D, Roberts JA, Owen R. The risks of medical complacency towards poliomyelitis. *Med J Aust*. 2020;213(2):61–3. doi: https://doi.org/10.5694/mja2.50681.