Australian National Enterovirus Reference Laboratory annual report, 2021

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# Abstract

Australia monitors its polio-free status by conducting surveillance for cases of acute flaccid paralysis (AFP) in children less than 15 years of age, as recommended by the World Health Organization (WHO). Cases of AFP in children are notified to the Australian Paediatric Surveillance Unit or the Paediatric Active Enhanced Disease Surveillance System, and faecal specimens are referred for virological investigation to the National Enterovirus Reference Laboratory. In 2021, no cases of poliomyelitis were reported from clinical surveillance and Australia reported 1.31 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 A4, coxsackievirus A10, coxsackievirus A13 and enterovirus A71 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 2021, there were five cases of wild poliovirus reported from the two remaining endemic countries: Afghanistan and Pakistan. Including Afghanistan and Pakistan, 22 countries also reported cases of AFP due to circulating vaccine-derived poliovirus.

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

# Introduction

Poliomyelitis is principally 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 18 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 that 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, so as 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 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, 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.

The number of wild poliovirus cases worldwide decreased significantly, from 140 in 2020, to just five cases in 2021; this is the lowest number of wild poliovirus cases ever recorded. Only wild poliovirus type 1 (WPV1) continues to be detected in the two remaining endemic countries, Afghanistan and Pakistan, which reported four cases and one case respectively in 2021.9 Global eradication of wild poliovirus types 2 and 3 was certified in 2015 and 2019 respectively.10 The full impact of the COVID-19 pandemic on poliovirus surveillance may not be known for some time; but if true, the small number of wild poliovirus cases reported in 2021 represents a genuine and tangible opportunity to achieve global eradication of wild poliovirus.11

Polio outbreaks due to circulating VDPV (cVDPV) can arise in areas where poor sanitation standards occur in conjunction with sustained low oral poliomyelitis vaccine (OPV) coverage. Although the number of cases of cVDPV declined in 2021 compared to 2020, cVDPV continues to present a challenge for the global polio eradication program. In 2021, cVDPV was detected in human and/or environmental samples in 33 countries, 29 of which were in the WHO African and Eastern Mediterranean Regions; greater than 95% of cases involved cVDPV type 2 (cVDPV2).12 Within the Western Pacific Region, cVDPV type 1 (cVDPV1) and cVDPV2 outbreaks were first detected in the Philippines in July 2019 and June 2019 respectively, with genetically-linked cases subsequently detected in both Malaysia and the Philippines; these outbreaks were declared over in June 2021 (Philippines) and September 2021 (Malaysia).13–15 Nevertheless, the WHO continues to list both countries as vulnerable to the emergence of further VDPV outbreaks. Recurrent cVDPV outbreaks highlight both the ongoing risks of transmission posed by wild poliovirus and cVDPV, and 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 2021, 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.16 For AFP cases involving children less than 15 years of age, paediatricians are requested to notify the NERL directly[[1]](#footnote-2) and to complete a clinical questionnaire.[[2]](#footnote-3),5 Designated nursing staff ascertain AFP cases from the medical records at the eight tertiary paediatric hospitals in which PAEDS operates.6 Duplicate notifications of AFP cases from both paediatricians and PAEDS staff can occur, but this 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, for faecal specimen collection to be classified as adequate.17 The faecal specimens are tested by virus culture at the NERL with funding from the Australian Government Department of Health.

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.[[3]](#footnote-4) 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.17 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 2021 equated to 48 cases based on the Australian Bureau of Statistics estimate of Australia’s population at 30 June 2020. 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 into 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, for between seven and 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 OPV strain, or a VDPV, in a process known as intratypic differentiation (ITD).20 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.21

## 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. Environmental samples are processed by the NERL according to the two-phase separation procedure published by the WHO.22 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 is 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 in the same manner 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, 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).

The NERL encourages members of the ERLNA to perform their own enterovirus typing. It has advised members of the ERLNA on enterovirus detection, has supplied laboratory and computer analysis protocols, and has performed tests in parallel with other laboratories for quality assurance purposes. Nevertheless, 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 varies from year to year, as does the number of results or referred specimens received by the NERL.

Clinical specimens are initially screened for enterovirus using a RT-qPCR assay directed to highly conserved genomic sequence in the 5’ untranslated region (5’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 2021, a total of 86 notifications of AFP cases were received (Table 1). Of these, 31 notifications were reported by the APSU surveillance system and 55 through PAEDS. Four notifications were deemed to be ineligible, due to the patient’s age being 15 years or older or because the clinical presentation was subsequently determined not to be AFP. Nineteen 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.

****Table 1: Notification of acute flaccid paralysis cases, 2021 by state and territory****

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| State or territorya | Estimated population aged < 15 yearsb | Expected number of AFP cases in 2021c | Total number of notifications | Ineligible notifications | Duplicate notifications | Eligible AFP cases with final classification by PEP | Non-polio AFP rate per 100,000 childrend |
| ACT | 82,775 | 1 | 0 | 0 | 0 | 0 | 0.00 |
| NSW | 1,509,265 | 15 | 33 | 1 | 4 | 28 | 1.87 |
| NT | 52,525 | 1 | 3 | 0 | 1 | 2 | 2.00 |
| Qld | 999,268 | 10 | 18 | 0 | 8 | 10 | 1.00 |
| SA | 310,041 | 3 | 2 | 0 | 0 | 2 | 0.67 |
| Tas. | 94,289 | 1 | 5 | 3 | 1 | 1 | 1.00 |
| Vic. | 1,216,321 | 12 | 16 | 0 | 3 | 13 | 1.08 |
| WA | 517,381 | 5 | 9 | 0 | 2 | 7 | 1.40 |
| **Australia** | **4,781,865** | **48** | **86** | **4** | **19** | **63** | **1.31** |

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 2020. 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.

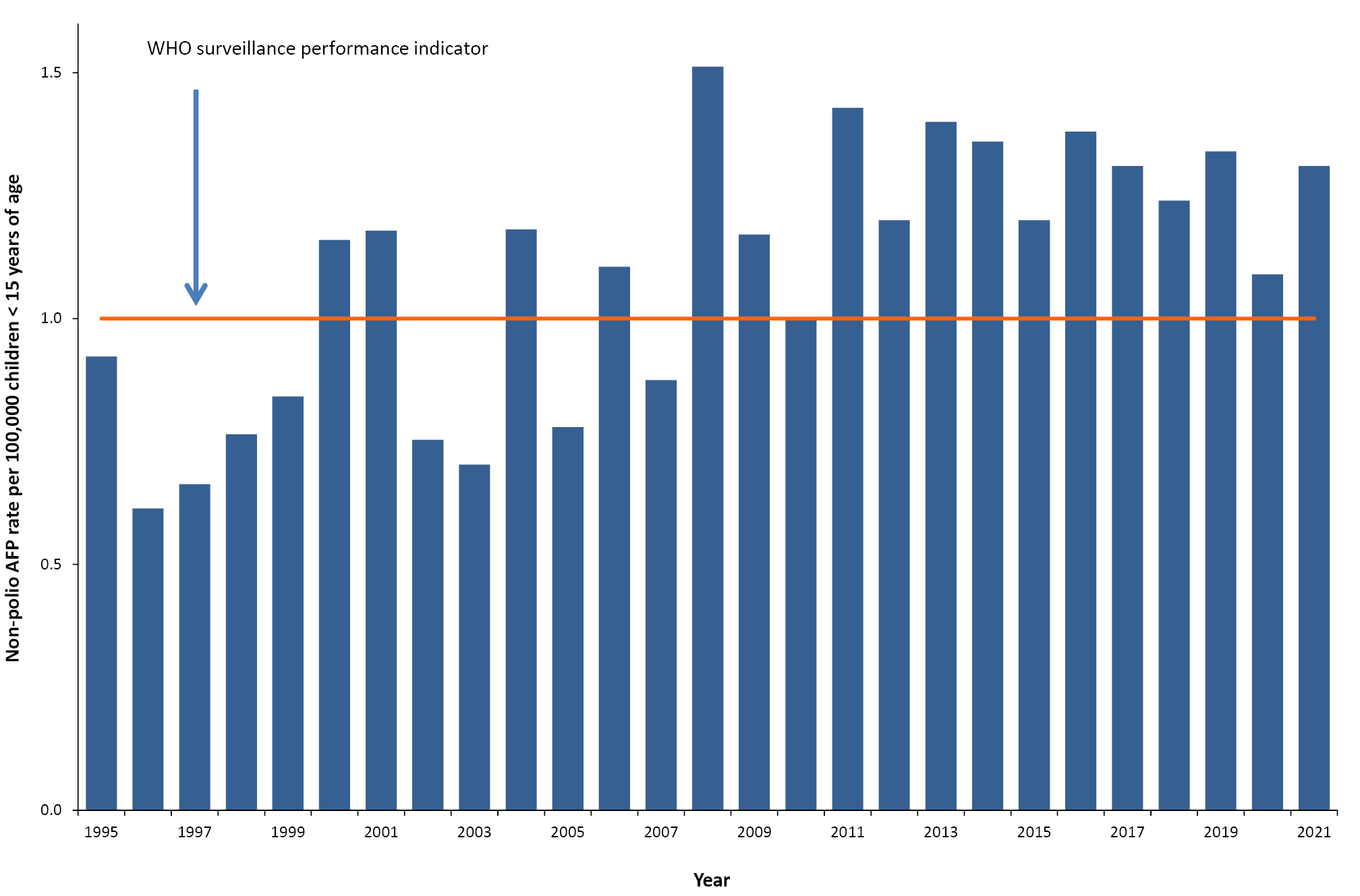
The PEP classified 63 cases as non-polio AFP, a rate of 1.31 cases per 100,000 children less than 15 years of age, which met the WHO AFP surveillance performance criterion for a polio-free country of at least one case of non-polio AFP per 100,000 children (Table 2, Figure 1). This result marks the fourteenth consecutive year in which Australia has achieved the WHO AFP surveillance target.

****Table 2: Australia’s surveillance for cases of acute flaccid paralysis, 2021, 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 2021) | 63 cases classified as non-polio AFP | 1.31 (63/48) 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) | 39 AFP cases with adequate specimens collected | 62% (39/63) classified non-polio AFP cases with adequate specimens |

Of the 63 non-polio AFP cases: 14 cases were notified by clinicians through both the APSU and PAEDS systems; 41 cases were notified through the PAEDS system only; and eight cases were notified through the APSU system only, with five of these cases 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 2021, with the PEP classifying 19 and eight cases respectively, with these two conditions.

**Figure 1: Non-polio acute flaccid paralysis rate, Australia 1995 to 2021a**



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.

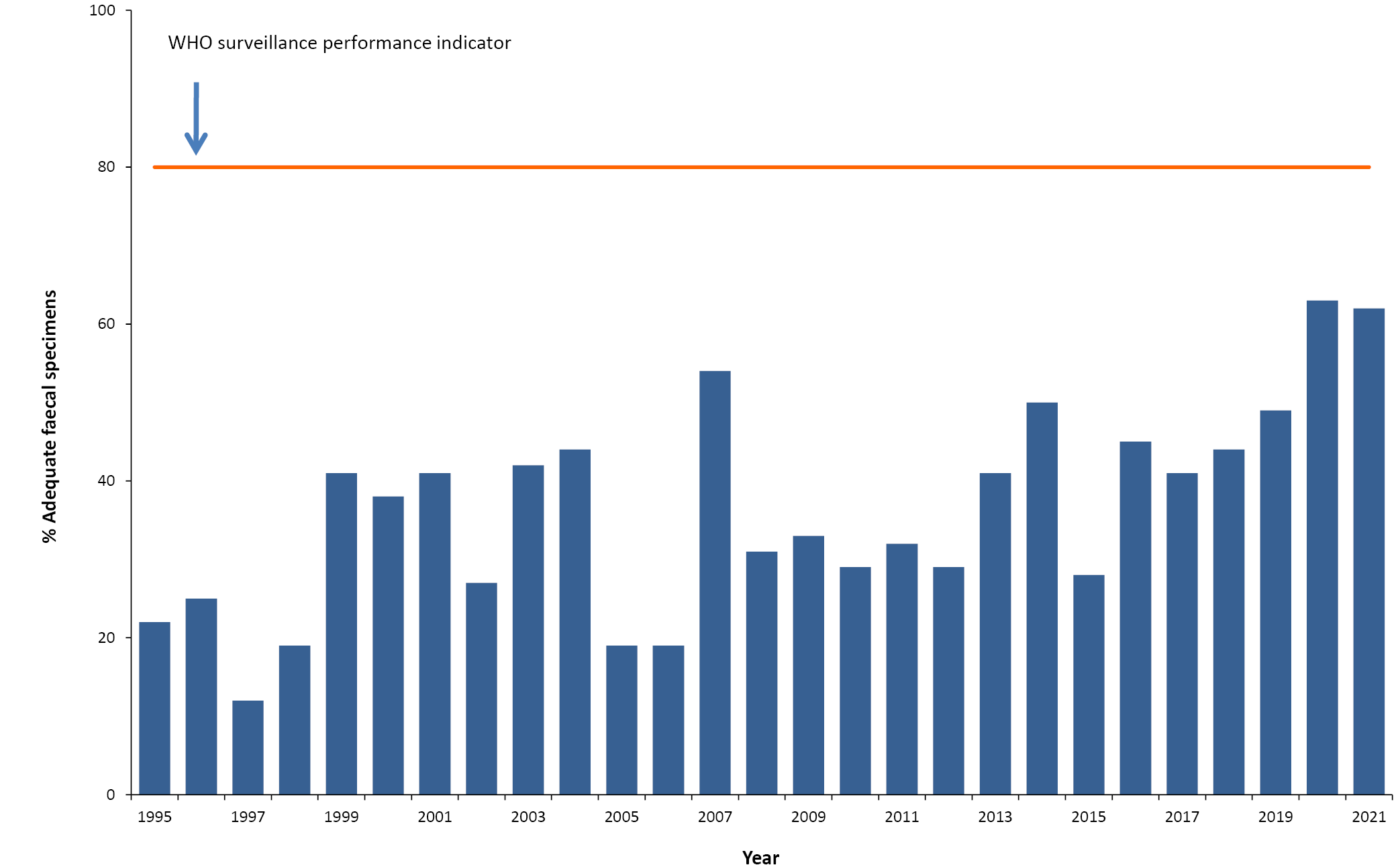
## Notification of AFP cases by state and territory

In 2021, AFP cases were notified from all jurisdictions in Australia except the Australian Capital Territory (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 New South Wales, the Northern Territory, Queensland, Tasmania, Victoria and Western Australia, with the Australian Capital Territory and South Australia the only jurisdictions not reaching the target.

## Faecal collection from AFP cases

In 2021, a total of 114 faecal specimens from 59 of the 63 eligible cases were tested at the NERL. Two specimens were collected from 39 of the eligible cases more than 24 hours apart and within 14 days of the onset of paralysis, satisfying the WHO criterion for adequate specimens and representing 62% of the non-polio AFP cases, compared to the WHO benchmark of 80% (Figure 2, Table 2). Although Australia has never attained this performance criterion, the percentage of adequate stools collected in 2020 (63%) and 2021 (62%) marked a significant improvement from previous years in which the proportion of adequate stools was frequently less than 50% (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 onset of paralysis; 78% of cases (49/63) had two specimens collected within this extended time frame.17

****Figure 2: Percentage of non-polio AFP cases with adequate faecal specimen collection, Australia 1995 to 2021a****



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.

Poliovirus was not detected in any of the specimens referred for AFP surveillance. The non-polio enteroviruses coxsackievirus A4 (n = 1), coxsackievirus A10 (n = 1), coxsackievirus A13 (n = 1) and EV-A71 (n = 2) were identified from stool specimens collected from five separate AFP cases: three in New South Wales; one in Queensland (coxsackievirus A13); and one in Victoria (EV-A71). Non-polio enteroviruses that, due to low viral load, could only be characterised as Enterovirus species were identified from stool specimens collected from another three AFP cases.

### Environmental surveillance

In 2021, the NERL tested 12 environmental samples with sample collection alternating between the Eastern and Western Treatment Plants in Melbourne. Poliovirus was not detected in any of these specimens. Non-polio enteroviruses were isolated from ten of the 12 environmental samples, with coxsackievirus B5 the most common enterovirus detected, identified in eight of the ten (80%) enterovirus-positive samples. Enterovirus infections are considered ubiquitous and the isolation of non-polio enteroviruses from environmental samples collected in polio-free countries not using OPV usually serves as an indicator of the quality of the sewage collection and test procedures.

### Enterovirus surveillance

In 2021, a total of 147 clinical specimens were referred to the NERL for enterovirus typing (Table 3). One hundred and twenty-eight specimens (87.1%) were referred from Victoria and 19 (12.9%) from interstate: 14 from the Australian Capital Territory; one from New South Wales; two from the Northern Territory; one from South Australia; and one from Tasmania. Of these specimens, 140 (95.2%) were characterised as non-polio enteroviruses, with 106 (72.1%) being fully typed and 34 (23.1%) characterised to the level of Enterovirus species only. Of the remaining specimens, one (0.7%) was characterised as rhinovirus and six (4.1%) were reported as no enterovirus identified (Table 3). Poliovirus was not detected in any of the specimens referred for enterovirus typing.

**Table 3: Laboratory results for Australian specimens reported by the NERL, 2021**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Result | Specimens from AFP cases involving children < 15 years of age | Specimens from AFP cases involving patients ≥ 15 years of age | Environmental surveillance | Enterovirus surveillance | Total |
| Rhinovirus | 1 | 0 | 0 | 1 | 2 |
| Non-polio enterovirus | 13 | 0 | 10 | 140 | 163 |
| No enterovirus identified | 102 | 5 | 2 | 6 | 115 |
| **Total** | **116** | **5** | **12** | **147** | **280** |

****Table 4: Enterovirus test results from samples originating in Australia, 1995 to 2021****

| Year | Poliovirus | | Non-polio enterovirus | No enterovirus detected | EVID results referreda | 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 |
| 1999b | 60 | 1 | 9 | 9 | 0 | 79 |
| 2000 | 45 | 0 | 44 | 47 | 0 | 136 |
| 2001b | 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 |
| 2007c | 0 | 2 | 32 | 115 | 107 | 256 |
| 2008 | 0 | 0 | 20 | 92 | 77 | 189 |
| 2009d | 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 |
| 2013e | 1 | 0 | 242 | 198 | 230 | 671 |
| 2014 | 0 | 0 | 68 | 128 | 506 | 702 |
| 2015f | 12 | 0 | 185 | 96 | 168 | 461 |
| 2016 | 0 | 0 | 242 | 143 | 227 | 612 |
| 2017g | 1 | 1 | 204 | 92 | 173 | 471 |
| 2018h | 2 | 0 | 231 | 89 | 198 | 520 |
| 2019i | 1 | 0 | 52 | 97 | 97 | 247 |
| 2020j | 1 | 0 | 91 | 135 | 20 | 247 |
| 2021 | 0 | 0 | 163 | 115 | 0 | 278 |

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.

In 2021, including specimens received for AFP and environmental surveillance, a total of 120 non-polio enteroviruses were typed and an additional 43 enteroviruses were characterised to the level of Enterovirus species only by the NERL (Table 3). Excluding rhinoviruses, a total of 278 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 2021 were coxsackievirus A6, coxsackievirus B5, and coxsackievirus A2.

Of note, there were 12 cases of EV-A71 detected in 2021, with two of the detections in AFP cases. The majority (9/12) of cases were detected in samples collected between December 2020 and March 2021. Nine of the cases were widely dispersed across metropolitan Melbourne, with one case from regional Victoria, one case from New South Wales, and one case from South Australia. The cases involved seven infants less than three months old, four children less than four years old, and a 24-year old. Six cases were identified from cerebrospinal fluid, with four presenting with fever. Another five cases were reported from faeces and one from swabs of hand, foot and mouth disease, which is the most common presentation of EV-A71 infection. All of the EV-A71 viruses detected belonged to the C6/C1-like sub-genogroup.

### Polio regional reference laboratory activities

In 2021, as part of its role as a Polio Regional Reference Laboratory, the NERL received two stool specimens from an AFP case in Brunei Darussalam and 32 specimens from AFP cases in Pacific Island countries, comprising 21 specimens from Fiji, nine from Solomon Islands, and two from Tonga. Coxsackievirus A13 was detected in two specimens from an AFP case from Solomon Islands and an enterovirus that could only be characterised as Enterovirus species was detected in a specimen from an AFP case from Fiji. Poliovirus was not isolated from any of the specimens.

A total of 94 stool specimens were received from Papua New Guinea and tested by the NERL, including 83 specimens from AFP cases involving children less than 15 years of age, eight from AFP cases aged 15 years or older, and three from contacts of AFP cases. Sabin-like poliovirus type 3 was identified in two specimens, each from a separate AFP case, indicative of recent vaccination with OPV. Importantly, cVDPV1 was not detected in any of the specimens tested in 2021, with the last detection of cVDPV1 linked to the 2018 outbreak from an environmental sample collected in November 2018.

In 2021, the NERL continued to provide laboratory support to Malaysia as part of the regional response to outbreaks of cVDPV1 and cVDPV2 that were first detected in the Philippines in 2019. A total of 18 isolates from environmental surveillance samples collected in Malaysia were referred to the NERL for sequencing and analysis of the poliovirus VP1 genomic region. Thirteen of the isolates were characterised as Sabin-like type 2 consistent with vaccination activities as part of the polio outbreak response. The outbreaks were subsequently declared over in June (Philippines) and September (Malaysia) 2021, with the last cases of cVDPV detected in sewage samples collected in January (Philippines) and March (Malaysia) 2020.12–15

### Quality assurance programs

In 2021, the NERL maintained its accreditation as a WHO Polio Regional Reference Laboratory through the successful completion of annual WHO quality assurance panels for poliovirus intratypic differentiation 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.

# Discussion

In 2021, Australia reported a non-polio AFP rate of 1.31 cases per 100,000 children less than 15 years of age, meeting the WHO AFP surveillance target for the fourteenth 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 circulating wild poliovirus or VDPV. Nevertheless, gaps in AFP surveillance were noted at the sub-national level with the Australian Capital Territory and South Australia failing to meet the WHO surveillance target.

Australia has never achieved the strict WHO surveillance target for adequate stool collection from 80% of non-polio AFP cases.24 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% and 62% of cases with adequate specimens in 2020 and 2021 respectively; these are 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 continue to be discussed at all 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 is considered the maximum duration of poliovirus shedding, 78% of AFP cases in 2021 had two specimens collected within this extended time frame.17

Poliovirus was not detected in any of the specimens referred for AFP surveillance or enterovirus typing in 2021. Non-polio enteroviruses EV-A71 and EV-D68 are commonly regarded as enteroviruses of public health concern due to their association with neurological disease and outbreaks.7,8 In this regard, it is noteworthy that 12 cases of EV-A71 were detected in 2021, with ten cases identified through enterovirus surveillance and two cases through AFP surveillance. While genetic sequencing confirmed all cases as belonging to the C6/C1-like sub-genogroup that clustered by phylogenetic analysis, the widespread distribution of cases, predominantly across metropolitan Melbourne, did not support a common transmission link.

Identification of increased detections of an enterovirus of public health significance through enterovirus surveillance highlights the merits of this program in complementing the clinical surveillance program focused on AFP cases. One limitation of the enterovirus surveillance program is that the surveillance network is a voluntary and passive system. In 2021, fewer than 15% of specimens referred for enterovirus typing were received from outside of Victoria, providing a limited picture of non-polio enterovirus circulation in other Australian jurisdictions.

With only five cases recorded for the year, 2021 saw the lowest number of wild poliovirus cases ever recorded and raised hopes that the global eradication of poliovirus is an attainable objective. Only WPV1 continues to be detected in the two remaining endemic countries: Afghanistan and Pakistan.9 Despite the ongoing COVID-19 pandemic, countries and partners of the Global Polio Eradication Initiative intensified their efforts to eradicate poliovirus, including the resumption of nationwide polio immunization campaigns across Afghanistan for the first time in more than three years.25 Nevertheless, WPV1 continues to be detected, particularly in Pakistan, in environmental samples collected as recently as December 2021, and significantly, WPV1 was recently detected in stool samples collected in November 2021 from a child in Malawi.9,26 This marks the first case of wild poliovirus detected in Africa in more than five years, with nucleotide sequence analysis confirming the virus to be genetically linked to isolates from Sindh Province in Pakistan.26 This detection highlights the continued risk of poliovirus importation into other countries as long as wild poliovirus continues to circulate.

Additionally, the number of countries reporting cVDPV outbreaks remains concerning. The worldwide removal of poliovirus type 2 from OPV in 2015, along with the introduction of at least one dose of trivalent inactivated polio vaccine in the routine immunisation schedules of all countries to maintain immunity to poliovirus type 2, was predicted to reduce the likelihood of cVDPV2 outbreaks. Yet, between January 2020 and June 2021, 86% of cVDPV outbreaks were type 2; and in every year since 2017, more cases of polio have been caused globally by cVDPV2 than by wild poliovirus.27,28 Although the genetic sequence of some of the cVDPV2 outbreaks has indicated that the origin of the virus lineage existed prior to the switch to bivalent OPV in 2015, other outbreaks are new emergences in countries adjoining those that used monovalent OPV2 in response to their own more recent cVDPV2 outbreaks.27,29

Given the urgent need to address recurrent cVDPV2 outbreaks, two novel OPV2 (nOPV2) vaccine candidates were developed that are more genetically stable and thus less capable of reversion to neurovirulence than is the original Sabin OPV2 strain they were based on. Clinical trial data demonstrated both nOPV2 candidate strains to be well tolerated with no serious adverse events, while providing comparable protection against poliovirus type 2.30 Emergency Use Listing of nOPV2 vaccine has been granted and nOPV2 is currently being used in response to a number of cVDPV2 outbreaks. In anticipation of the use of nOPV2 in the field, WHO released an updated version of the ITD assay to enable detection and differentiation of wild, Sabin-like and nOPV type 2 poliovirus strains.

The Australian Government Department of Health developed a methodology to calculate the risk to Australia’s health security if there was a polio outbreak.31 In 2019, there was assessed a very low risk of importation of wild poliovirus or vaccine-derived poliovirus and occurrence of a resultant outbreak from sustained transmission in Australia from 2019 to 2023. The international response to the Papua New Guinea cVDPV1 outbreak was still proceeding when the risk assessment was first performed in 2019, but more recent polio outbreaks in Malaysia and the Philippines have demonstrated the need to perform the national polio risk assessment regularly and as new outbreaks occur.

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