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Australian National Enterovirus Reference Laboratory annual report, 2020

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Annual report

Australian National Enterovirus Reference Laboratory annual report, 2020

Matthew B Kaye, Arnau Garcia-Clapes, Linda K Hobday, Aishah Ibrahim, Presa Chanthalavanh, 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 2020, no cases of poliomyelitis were reported from clinical surveillance; Australia reported 1.09 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 A10 and coxsackievirus A16 were identified from clinical specimens collected from AFP cases. Australia also performs enterovirus surveillance and environmental surveillance to complement the clinical system focussed on children. In 2020, there were 140 cases of wild poliovirus reported from the two remaining endemic countries: Afghanistan and Pakistan. Another 28 countries reported cases of 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 less 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 at least 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 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 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 from 176 in 2019 to 140 in 2020, with only wild poliovirus type 1 detected in Afghanistan and Pakistan, reporting 56 and 84 cases respectively. Nonetheless, the full impact the COVID-19 pandemic had on poliovirus surveillance in 2020 may not be known for some time.[9,10] The global eradication of wild poliovirus types 2 and 3 was certified in 2015 and 2019 respectively.[11] In August 2020, four years after the last wild poliovirus type 1 case was reported in Nigeria, the WHO African Region was certified as wild-poliovirus-free.[12] With this certification, five of the six WHO regions, representing over 90% of the world's population, are now free of wild poliovirus.

Polio outbreaks due to VDPV can arise in areas with sustained low oral polio vaccine coverage. In 2020, circulating VDPV (cVDPV) types 1 and/or 2 were reported in 30 countries, 27 of which were in the WHO African and Eastern Mediterranean Regions.[13] Within the Western Pacific Region, both the Philippines and Malaysia were affected by genetically-linked cVDPV outbreaks, with the first cases detected in the Philippines in June (cVDPV2) and July (cVDPV1) 2019. Subsequently cVDPV1 and cVDPV2 isolates were detected in AFP cases and/or environmental sewage samples collected in both countries. Recurrent cVDPV outbreaks highlight the ongoing risk of transmission posed by both 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 polio has been certified.

This report summarises the poliovirus surveillance program in Australia for 2020, 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.[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.[5,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

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

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

from both paediatricians and PAEDS staff can occur, but represent a sensitive surveillance system. Duplicate notifications are excluded from data analysis.

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.[15] 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.[iii] Ineligible cases are not reported to the WHO.

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

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.[15] 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 2020 equated to 47 cases based on the Australian Bureau of Statistics estimate of Australia's population at 30 June 2019. 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 wild poliovirus or cVDPV cases if poliovirus is circulating.

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).[16,17] 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).

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 poliomyelitis vaccine (OPV) strain (Sabin-like),

a novel oral poliomyelitis vaccine type 2 (nOPV2) strain or a VDPV, in a process known as intratypic differentiation (ITD).[18] 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 to rapidly determine the likely source of the virus, as utilised in the 2007 wild poliovirus importation in Australia.[19]

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.[20] In brief, 800 ml of sewage is collected as a grab sample prior to any biological or chemical treatment. At the laboratory 500 ml of the sample is vigorously shaken at 4°C with dextran, polyethylene glycol and sodium chloride. The mixture is incubated overnight at 4°C in a separating funnel and the lower organic phase collected the next day and clarified using chloroform treatment and centrifugation. The sample extract is inoculated onto L20B and RD-A cell lines and observed microscopically for cytopathic effect as for faecal specimens. All enterovirus isolates from cell culture are typed by nucleic acid sequencing as described in the 'enterovirus surveillance' section below.

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 nine public sector diagnostic virology laboratories in the Australian Capital Territory (Canberra Hospital), New South Wales (Royal Prince Alfred Hospital and the Institute of Clinical Pathology and Medical Research), Queensland (Queensland Health and Scientific Services), South Australia (SA Pathology), Tasmania (Royal Hobart Hospital), Victoria (Royal Children's Hospital and VIDRL) and Western Australia (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. The NERL receives un-typed enteroviruses from two laboratories for typing on a regular basis.

Clinical specimens are initially screened for enterovirus using a RT-qPCR assay directed to highly-conserved sequence in the 5' untranslated region (UTR).[21] 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 employed to characterise the enterovirus to the species level only and may exclude poliovirus.

Results

Classification of AFP cases

In 2020, a total of 71 notifications of AFP cases were received, with 26 cases reported by the APSU surveillance system and 45 through PAEDS (Table 1). The PEP classified 51 cases as non-polio AFP, a rate of 1.09 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).

Table 1: Notification of acute flaccid paralysis cases, 2020 by state and territory

State or territory	Estimated population aged < 15 years[a]	Expected number of AFP cases in 2020[b]	Total number of notifications	Ineligible notifications	Duplicate notifications	Eligible AFP cases with final classification by PEP	Non-polio AFP rate per 100,000 children[c]
ACT	81,390	0.5	0	0	0	0	0.00
NSW	1,499,863	15.0	21	0	0	21	1.40
NT	52,840	0.5	1	0	0	1	2.00
Qld	989,916	10.0	24	2	9	13	1.30
SA	308,953	3.0	2	0	0	2	0.67
Tas.	94,123	1.0	0	0	0	0	0.00
Vic.	1,201,923	12.0	21	5	4	12	1.00
WA	511,842	5.0	2	0	0	2	0.40
Australia	**4,740,850**	**47**	**71**	**7**	**13**	**51**	**1.09**

a Australian Bureau of Statistics, estimated population at 30 June 2019. Available at https://www.abs.gov.au.
b 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. Some jurisdictions are not expected to report an AFP case each year based on the population < 15 years old.
c 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, 2020, 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 (47 cases for Australia in 2020)	51 cases classified as non-polio AFP	1.09 (51/47) non-polio AFP cases per 100,000 children < 15 years
≥ 80% of classified AFP cases with adequate specimens (two faecal specimens collected at least 24 hours apart and within 14 days of onset of paralysis)	32 AFP cases with adequate specimens collected	63% (32/51) classified non-polio AFP cases with adequate specimens

Figure 1: Non-polio acute flaccid paralysis rate, Australia 1995 to 2020[a]

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

This result marks the thirteenth consecutive year in which Australia has achieved the WHO AFP surveillance target. Six of the non-polio AFP cases were notified through APSU by clinicians at hospitals where PAEDS does not operate.

Guillain-Barré syndrome and transverse myelitis were the most common causes of non-polio AFP in 2020, with the PEP classifying 11 cases of each condition. Thirteen cases were notified by more than one source, whether by two or more clinicians through the APSU or by a clinician and the PAEDS system. Seven notifications were deemed to be ineligible due to the patient's age being greater than 14 years or for which the clinical presentation was subsequently determined not to be AFP.

Notification of AFP cases by state and territory

In 2020, AFP cases were notified from all jurisdictions in Australia except the Australian Capital Territory and Tasmania (Table 1). The non-polio AFP rates for eligible cases met the WHO AFP surveillance performance indicator of at least one case per 100,000 children less than 15 years of age in New South Wales, Northern Territory, Queensland and Victoria, with the Australian Capital Territory, South Australia, Tasmania and Western Australia not reaching the target.

Faecal collection from AFP cases

In 2020, a total of 95 faecal specimens from 44 of the 51 eligible cases were tested at the NERL. Two specimens were collected from 32 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 63% 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 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 still be detected after 60 days; 86% of cases had at least one specimen collected within this extended time frame.[14] Poliovirus was not detected in any of the specimens. The non-polio enteroviruses coxsackievirus A10 and coxsackievirus A16 were identified from stool specimens collected from two separate AFP cases, both in New South Wales.

Environmental surveillance

In 2020, the NERL tested 16 environmental samples with sewage collected at the Eastern (n = 7) and Western (n = 9) Treatment Plants in Melbourne. A poliovirus type 3 was isolated from one sample from the Western Treatment Plant collected in January 2020 and the nucleotide sequence of the VP1 region had 99.89% identity to the prototype Sabin vaccine strain, indicative of a recent vaccination event. Enteroviruses were not detected in 13 of 14 environmental samples collected between September and December 2020. 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.

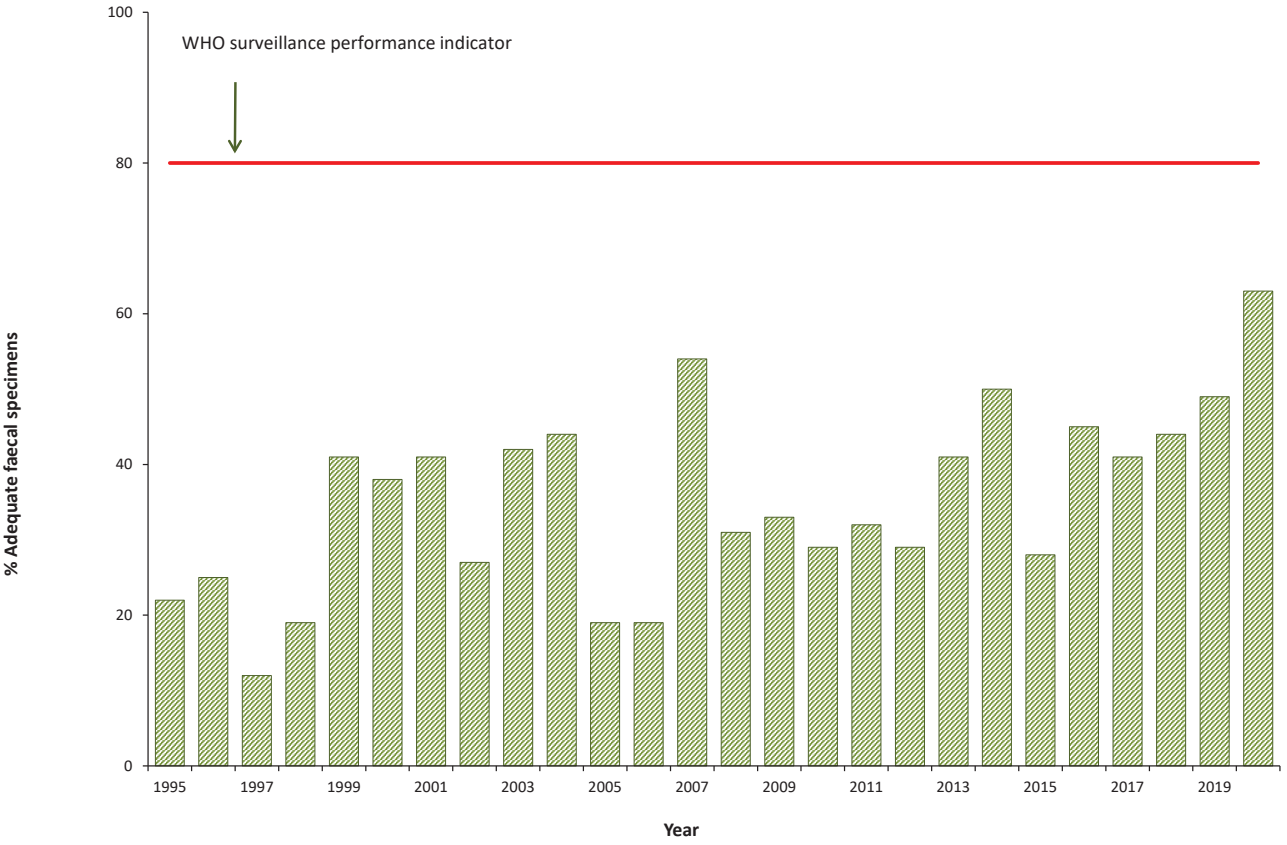
Enterovirus surveillance

In 2020, a total of 110 clinical specimens were referred to the NERL for enterovirus typing (Table 3). Of these specimens, 82 (75%) were characterised as non-polio enteroviruses, 4 (4%) as rhinovirus and 24 (22%) were reported as no enterovirus identified (Table 3). Poliovirus was not detected in any of the specimens referred for enterovirus typing. Including specimens received for AFP and environmental surveillance, a total of 54 non-polio enteroviruses were typed and an additional 37 enteroviruses were characterised to the species level only by the NERL (Table 3). An additional 20 non-polio enteroviruses were typed from clinical specimens by 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 2020 were coxsackievirus A6, coxsackievirus B5, and echovirus 18.

Polio regional reference laboratory activities

In 2020, 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 18 specimens from AFP cases in Pacific Island countries, including 12 from Fiji and two each from the Solomon Islands, Tonga and Vanuatu. Poliovirus was not isolated, nor was enterovirus detected, in any of these specimens.

Figure 2: Adequate faecal specimen collection rate, Australia 1995 to 2020[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 highlighted by the red line.

Table 3: Laboratory results for Australian specimens reported by the NERL, 2020

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	Total
Sabin poliovirus type 3	0	0	1	0	1
Rhinovirus	1	0	0	4	5
Non-polio enterovirus	5	0	4	82	91
No enterovirus identified	89	10	12	24	135
Total	95	10	17	110	232

a A total of 16 environmental samples were tested, with Sabin poliovirus type 3 and a non-polio enterovirus both detected in the same sample.

Table 4: Enterovirus test results from samples originating in Australia, 1995 to 2020

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[i]	1	0	91	135	20	247

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 Two separate isolations of Sabin-like poliovirus type 3 (one in 2019 and one in 2020) were identified from sewage.

A total of 151 stool specimens were received from Papua New Guinea and tested by the NERL, including 139 specimens from AFP cases involving children less than 15 years of age, ten from AFP cases greater than 14 years of age and two from contacts of AFP cases. Sabin-like poliovirus types 1 and/or 3 were identified in 14 (9.3%) of these specimens, indicative of recent vaccination with OPV. Importantly, VDPV1 was not detected in any of the specimens tested in 2020, with the last detection of cVDPV1 from an environmental sample collected in November 2018.

In 2020, the NERL provided laboratory support to Malaysia as part of the regional response to an outbreak of cVDPV types 1 and 2 by sequencing the VP1 region of poliovirus isolates from AFP and environmental surveillance. A total of 30 isolates from AFP cases, 109 isolates from environmental samples and 50 isolates from healthy individuals were received for testing. Six cVDPV1 isolates were characterised from AFP cases and 36 cVDPV1 and/or cVDPV2 isolates were characterised from environmental samples. Poliovirus isolates from the 50 healthy individuals were all characterised as Sabin-like consistent with vaccination activities as part of the polio outbreak response.

Quality assurance programs

In 2020, the NERL maintained its accreditation as a WHO Polio Regional Reference Laboratory through the successful completion of annual WHO quality assurance panels for poliovirus isolation and intratypic differentiation. The NERL also successfully participated in a WHO pilot panel for environmental surveillance; 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

In 2020, Australia reported a non-polio AFP rate of 1.09 cases per 100,000 children less than 15 years of age, meeting the WHO AFP surveillance target for the 13th 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, South Australia, Tasmania and Western Australia failing to meet the WHO surveillance target.

The submission of paper questionnaires through the APSU surveillance system limited the timely reporting of clinical information. From July 2020, an online portal to submit AFP clinical questionnaires was made available[iv] and is expected to facilitate the timely notification and ascertainment of AFP case histories through improved accessibility and ease of completion.

Australia has never achieved the strict WHO surveillance target for adequate stool collection from 80% of non-polio AFP cases.[22] In 2020, the PAEDS network implemented an action plan to improve the rate of adequate stool collection from AFP cases, which was a significant factor in Australia reporting 63% of cases with adequate specimens in 2020, the highest level reported since AFP surveillance was established in 1995. Furthermore, 86% of AFP cases in 2020 had at least one specimen collected within 60 days after the onset of paralysis, which is considered to be the maximum duration of poliovirus shedding.[14]

The Australian Government Department of Health developed a methodology to calculate the risk to Australia's health security if there was a polio outbreak.[23] In 2019, the risk—of wild poliovirus or vaccine-derived poliovirus

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

importation, resulting in an outbreak from sustained transmission in Australia from 2019 to 2023—was assessed to be very low. The international response to the Papua New Guinea cVDPV1 outbreak was still proceeding when the risk assessment was 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.

Enterovirus and environmental surveillance supplement the AFP surveillance program, providing additional means of monitoring Australia's polio-free status. A Sabin-like poliovirus type 3 isolate was reported from a sewage sample collected in Melbourne in 2020. Genetic sequencing determined the virus to be a Sabin-like poliovirus that was likely to have been shed by a visitor or returned traveller from a country that still uses the live attenuated oral polio vaccine, because Australia has exclusively used the inactivated polio vaccine since late 2005.

It is noteworthy that in 2020, enteroviruses were not detected in 13 of the 16 environmental samples tested at the NERL. This observation was supported by a decreased incidence of enterovirus and norovirus infections during the second lockdown period in Victoria in response to the COVID-19 pandemic and was similar to a significant decrease in seasonal influenza activity in Australia in 2020.[24,25] The reduced incidence of enteroviruses and other infectious pathogens during the COVID-19 pandemic was likely a result of the widespread implementation of community mitigation measures including restricting travel to close to home, school closures, social distancing in public, wearing of face masks and awareness of personal hand hygiene as well as the closure of domestic and international borders.

While Afghanistan and Pakistan remain the only polio-endemic countries with ongoing transmission of wild poliovirus type 1, the number of countries reporting cVDPV outbreaks is 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. Although the genetic sequence of some of the cVDPV2 outbreaks indicated the origin of the virus lineage existed prior to the switch to bivalent OPV in 2015, other instances are new emergences in countries adjoining those that used monovalent OPV2 in response to their own more recent cVDPV2 outbreaks.[26]

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 compared to the original Sabin OPV2 strain on which they were based. 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.[27] Emergency Use Listing of nOPV2 vaccine has been granted and is expected to be used in response to cVDPV2 outbreaks from 2021. 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.

With the eradication of two of the three wild poliovirus strains certified, the global polio eradication program has entered the endgame phase. In 2015, WHO member states committed to only retaining polioviruses in poliovirus-essential facilities that are certified to stringent containment conditions, so as to minimise the risk of poliovirus being reintroduced to the community through a containment breach.[28] Facilities that retain clinical and environmental samples and their derivatives may also be inadvertently storing poliovirus if the original material was collected while wild poliovirus or VDPVs were circulating or while OPV was in use, whether the material was from Australia or imported from overseas. To identify such facilities, the Australian Government Department

of Health commissioned the CSIRO to conduct the 2021 Australian Facilities Survey for the management of polioviruses and poliovirus potentially-infectious materials. Every facility holding known stocks of poliovirus, or which could hold items that are poliovirus potentially-infectious material, is requested to complete the survey.[v] Ensuring that the laboratory containment of polioviruses is restricted to poliovirus-essential facilities will contribute to the achievement of global polio eradication.

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