Report of the Australian National Polio Reference Laboratory: 1 January to 30 June 2000

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Abstract

The Australian National Polio Reference Laboratory (NPRL) is responsible for virological confirmation of poliomyelitis in Australia. Since 1995, 1,204 untyped enterovirus or poliovirus isolates from six States have been identified and characterised. Of these, 666 were Sabin vaccine-like poliovirus, 498 were non-polio enteroviruses and 39 were other viruses or negative; one non-Sabin vaccine-like poliovirus was identified as a laboratory contaminant. Early in 2000, the Victorian Infectious Diseases Reference Laboratory (VIDRL) was funded to coordinate surveillance of acute flaccid paralysis (AFP). From 1 January to 30 June 2000, 23 specimens from 13 patients with AFP were processed and cultured for the presence of enterovirus; none was detected. A National Coordinator has been appointed to work with the VIDRL and the Commonwealth Department of Health and Aged Care (CDHAC) to implement the Australian component of the World Health Organization's global plan for containment of wild polioviruses. During April 2000 staff of the NPRL and CDHAC met with the Regional Commission and staff of the World Health Organization (WHO) office of the Western Pacific Region (WPR) to discuss documentation required to certify Australia as poliovirus free. *Commun Dis Intell* 2000;24:300-303.

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Introduction

In order for the Western Pacific Region (WPR)* of the World Health Organization (WHO) to be declared wild poliovirus free, each country in the WPR must demonstrate high quality surveillance of acute flaccid paralysis (AFP), good polio vaccine coverage, an outbreak response plan and the containment of laboratory-sourced wild poliovirus.

The National Certification Committee (NCC) provides to the Regional Certification Commission (RCC) documentation on national surveillance and immunisation data, and an annual summary of the progress towards certification. The laboratory component is an essential part of the documentation process. The National Coordinator of Poliovirus Containment in Australia located at the Victorian Infectious Diseases Reference Laboratory (VIDRL) is working with the Australian National Poliovirus Reference Laboratory (NPRL) and the Commonwealth Department of Health and Aged Care (CDHAC) to develop and implement a national plan for containment of wild poliovirus infectious materials.

Surveillance of AFP began in Australia in March 1995 and is used for detection of wild poliovirus in the community. Based on experience of other countries, there should be one AFP case per annum for every 100,000 children below 15 years of age. In collaboration with the Australian Paediatric Surveillance Unit (APSU) and the VIDRL, AFP surveillance was coordinated by staff of the National Centre for Disease Control at the CDHAC from 1995 to early 2000, when the coordination was transferred to the VIDRL.

As well as AFP surveillance, another approach to detecting wild polioviruses is to characterise all polioviruses isolated from all patients regardless of their illness or age. In a country where oral poliovirus vaccine (OPV) is administered there will always be incidental isolations of OPV strains. To ensure that all polioviruses are identified and subsequently characterised as wild or Sabin vaccine-like, the NPRL tests polioviruses and untyped enteroviruses referred from virology laboratories in all Australian States. Earlier reports on activities of the NPRL for 1998[1] and the two halves of 1999[2,3] have been published. This report is for the period 1 January to 30 June 2000.

Methods and results

Certification

The Australian National Poliovirus Certification Committee (ANPCC) has addressed the important issue of containment of wild poliovirus infectious and potentially infectious materials. For Australia's submission to the Regional Certification Commission (RCC) the NPRL has documented the history of poliomyelitis in Australia, and reported on

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* Abbreviations: AFP, Acute Flaccid Paralysis; ANPCC, Australian National Poliovirus Certification Committee; APSU, Australian Paediatric Surveillance Unit; CDC, Centers for Disease Control and Prevention, Atlanta; CDHAC, Commonwealth Department of Health and Aged Care; NAPH, nucleic acid probe hybridisation; NCC, National Certification Committee; NPRL, National Polio Reference Laboratory; RCC, Regional Certification Committee; RT-PCR, Reverse Transcriptase Polymerase Chain Reaction; OPV, oral poliovirus vaccine; VIDRL, Victorian Infectious Diseases Reference Laboratory; WHO, World Health Organization; WPR, Western Pacific Region.

laboratory findings and laboratory containment of wild poliovirus.

AFP surveillance

Paediatricians are requested to report all cases of AFP to the APSU by mail, and to contact the national coordinator of AFP surveillance without delay. Arrangements are made for two faecal samples to be collected at least 24 hours apart and within 14 days of onset of paralysis. The samples are transported to the NPRL at the VIDRL for testing. In order to investigate the case at its early phase a questionnaire is sent to the paediatrician; after 60 days another is sent to obtain follow-up information on the patient's condition. The Polio Expert Committee reviews the completed questionnaires and laboratory findings on all reported cases, and classifies them using the WHO virological classification.[4]

Within 24 hours of receipt, faecal samples are processed and inoculated into six different cell lines including the two cell lines (RD and L20B) recommended by the WHO. The L20B cells possess receptors for human poliovirus and are used to select poliovirus. RD cells support the growth of various enteroviruses including poliovirus.

In all 23 faecal samples from 13 patients presenting with AFP were sent to the NPRL during the first 6 months of 2000 (Table 1). Seven samples were dispatched to the NPRL within the recommended 3 days after collection, eleven were sent after 3 to 7 days and five after 8 to 14 days. Six patients were in Queensland, three in New South Wales and two each in Victoria and South Australia. Of these, six patients had two samples collected at least 24 hours apart within 14 days of onset of illness. One patient had two samples collected only 12 hours apart but still within the recommended 14 days of paralysis and another patient had a single sample collected more than 14 days after onset. Onset dates were not available for five patients, two of whom had single samples collected. No virus was detected in any of these 23 samples.

Table 1. Testing of specimens for enteroviruses from patients with acute flaccid paralysis, Australia, 1 January to 30 June 2000

State/ Territory	District/city	Specimen date (& time)	Result
Qld	Kippa Ring	17.1.00 (0700)	Negative
		17.1.00 (1900)	Negative
Vic	Bundoora	24.1.00	Negative
		27.1.00	Negative
Qld	Yeppoon	1.2.00	Negative
SA	Klemzig	18.2.00	Negative
Qld	Caboolture	4.3.00	Negative
		5.3.00	Negative
Qld	Oakey	28.3.00	Negative
SA	Hackham West	28.4.00	Negative
		1.5.00	Negative
NSW	Kurri Kurri	3.5.00	Negative
		4.5.00	Negative
ACT	Canberra	17.5.00	Negative
		18.5.00	Negative
Qld	Cairns	21.5.00	Negative
		23.5.00	Negative
Qld	Biggenden	22.5.00	Negative
		25.5.00	Negative
NSW	Bonnells Bay	5.6.00	Negative
		7.6.00	Negative
Vic	Brunswick	24.6.00	Negative
		25.6.00	Negative

Testing of referred specimens

One faecal sample from a 74-year old female who had diarrhoea and paralysis was processed and cultured for enterovirus. No virus was isolated.

Poliovirus antibody test by neutralisation was performed on a single serum sample from a 3-year old female with an unknown immunity status. Antibodies to all three strains of poliovirus were detected suggestive of past immunisation or infection.

In January 2000, four faecal samples from two boys with AFP aged 1 and 4 years were referred to VIDRL from the WHO Infectious Disease Surveillance Unit, East Timor. An enterovirus isolated from both specimens collected from the 4-year old boy was identified as Coxsackie A24 using molecular (reverse transcriptase polymerase chain reaction; RT-PCR) and sequencing methods with primers supplied by the Enterovirus Laboratory, Centers for Disease Control (CDC) and Prevention, Atlanta, USA. Neutralisation assay and immune electron microscopy confirmed the identity of both isolates. No virus was isolated from the one-year old patient.

Identification of referred enteroviruses and polioviruses

All poliovirus isolates were characterised using nucleic acid probe hybridization (NAPH). Those from AFP patients were also tested using enzyme immunoassay. Three were also characterised by the RT-PCR method using Pan Polio, Polio type specific and Sabin type specific primers supplied by the Enterovirus Laboratory, CDC.

Between 1 January and 30 June 2000, 128 polioviruses or untyped enteroviruses were referred to the laboratory. Eighty-nine (69%) isolated between 1994 to 2000 were referred from New South Wales and 36 (28%) isolated between 1991 and 2000 were sent from Western Australia. Three isolates (2%) were referred from Victoria. Of these 128 isolates, 106 (83%) were characterised as Sabin vaccine-like polioviruses. Fifteen (12%) could not be recovered in RD or L20B cells so were either another virus or were no longer viable. Seven (5%) were recovered in RD but not in L20B cells so were presumed non-polio enteroviruses and not identified further. Concordant results were obtained by both NAPH and RT-PCR.

Cumulative testing and results

Cumulative results of testing of polio and enterovirus isolates referred to the laboratory from within Australia are summarised in Table 2. This Table includes several isolates for previous years not previously reported by the NPRL; three States submitted these earlier this year as outlined above. Since 1995, 1,204 isolates have been tested at the VIDRL. Of these, 666 (55%) were identified as Sabin vaccine-like polioviruses, 498 (41%) as non-polio enteroviruses, and 39 (3%) as other viruses or, on the basis

Table 2. Cumulative summary of identification of enteroviruses and intratypic differentiation of polioviruses from Australian laboratories performed at the National Polio Reference Laboratory, 1 January 1995 to 30 June 2000

State	Year	Polio: Sabin-like	Non-polio enterovirus	Non-enterovirus negative	Total
Vic	1995	9			9
	1996	17			17
	1997	5			5
	1998	7			7
	1999	19			19
	2000	3			3
Qld	1995	41	5	8	54
	1996	99	4	9	112
	1997	41			41
	1998	8	15	2	25
	1999	2			2
W A	1995/6	133	384	5	522
	1997	32	76		108
	1998			2	2
	1999	3	9	9	21
	2000	4		4	8
Tas	1995	1			1
	1996	3			3
	1997	4			4
	1998	4			4
	1999	4			4
NSW	1994	5			5
	1995	76	5		81
	1996	35			35
	1997	39			39
	1998	30			31[#]
	1999	31			31
	2000	4			4
S A	1997	3			3
	1998	3			3
	1999	1			1
Total	1995-2000	666	498	39	1,204

[#] Includes one non-Sabin poliovirus type 2.

of failure to produce a cytopathic effect after 14 days' incubation, virus-negative.

One poliovirus isolate described in an earlier report[2] was characterised as non Sabin-like. It was identical to the non-Sabin attenuated poliovirus control used in the referring laboratory and was subsequently confirmed as a laboratory contaminant.

Containment of wild poliovirus

A national workshop to discuss Australia's approach to containment of wild poliovirus infectious and potentially infectious materials was held at the VIDRL in March 2000. A National Advisory Committee and representatives in each State and Territory assist the VIDRL and CDHAC staff in the containment process.

Currently a survey is being conducted nationally to identify those laboratories that may have wild poliovirus and potentially infectious material.[5] A national inventory will be presented to the WHO WPR office as part of the containment plan.

Other activities

As part of its reference role the VIDRL provides biological material to assist other laboratories. Between 1 January and 30 June 2000, the NPRL supplied enterovirus antisera and prototype enteroviruses and respiratory viruses to laboratories in Victoria and New South Wales. Reference Sabin poliovirus strains were supplied to laboratories in Western Australia and Victoria and cultures of RD, L20B and A549 cells to laboratories in Western Australia and New South Wales.

Discussion

Western Pacific Regional Certification

The WHO is firmly committed to poliomyelitis eradication globally by the end of the year 2000. Certification of each region requires the absence of wild poliovirus for at least 3 years in the presence of high quality surveillance. The last reported case of indigenous wild poliovirus in the WPR was in Cambodia in March 1977. All WPR countries are working to provide documentation so the WPR Certification Commission can assess whether the region should be certified as wild poliovirus free.

Australian certification

Staff of the CDHAC and NPRL have collated evidence of the absence of wild poliovirus for 3 years in the presence of high quality routine AFP surveillance among children under 15 years of age. In August 2000 the ANPCC met with the WPR RCC in Manila, produced documentation on the certification criteria and recommended that Australia be declared wild poliovirus-free.

Surveillance and investigation of acute flaccid paralysis

The APSU, CDHAC and NPRL have publicised the need for AFP surveillance and testing. Paediatricians enrolled with the APSU were sent flyers in January 2000 with instructions on reporting AFP patients and the collection and shipment of stool samples. Since February 2000 both surveillance and laboratory investigations of AFP have been administered by the NPRL leading to effective coordination of questionnaires, sample collection and transport.

Between 1 January and 30 June 2000, 23 samples from 13 patients were referred to NPRL for testing. Although this is below the optimal number for certification (20 patients Australia-wide; one per 100,000 children below 15 years of age per 6 months)[4] it is higher than for the same period last year.[2] Over the years the number of AFP samples referred to the NPRL has increased; samples from 4, 11 and 27 patients were referred to the NPRL in 1997, 1998 and 1999 respectively.

Continuous communication to highlight both the importance of AFP surveillance and the availability of information is critical to the eradication of wild poliovirus. An annual newsletter, a Poliovirus Homepage (which is continuously

updated) and six-monthly reports published in *Communicable Diseases Intelligence*[1,2,3] provide information.

Characterisation of polioviruses

Staff of the CDI Virology and Serology Laboratory Reporting Scheme (LabVISE) have urged all laboratories reporting polio and untyped enteroviruses to refer isolates to the NPRL. The majority of these samples have now been referred and all polioviruses isolated confirmed as Sabin vaccine-like.

Training in diagnostic polymerase chain reaction (PCR) techniques was provided in November 1999 to assist in identification and characterisation of polio and enteroviruses.[3] To date, the NPRL is accredited to characterise poliovirus isolates by NAPH and EIA. However, diagnostic PCR has been adapted in the NPRL as an additional test for characterisation of some poliovirus isolates.

The NPRL is currently seeking accreditation by the WHO for the use of PCR techniques for identification and intratypic differentiation of isolates. The NPRL is also attempting to sequence non-polio enteroviruses that cannot be typed by conventional neutralisation assays. These viruses may be variants of known enteroviruses or unassigned enteroviruses. As wild poliovirus is eradicated globally, these enteroviruses may become more prominent as causative agents of AFP.

Containment

In order for Australia to be certified as poliovirus free, it needs to demonstrate high quality AFP surveillance and be wild poliovirus free for at least 3 years. There are sufficient data to conclude that the last cases of indigenously acquired wild poliovirus infections occurred in Australia in 1972.[6]

Once circulation of wild poliovirus is successfully interrupted world wide, poliomyelitis will become the second infectious disease (after smallpox) to be eradicated. The only other reservoir of wild poliovirus will then be within laboratories. To prevent reintroduction of wild poliovirus into the community, containment of poliovirus in laboratories is essential.[7] A comprehensive report on Australia's approach to laboratory containment of wild poliovirus has been described in an earlier paper.[5] A plan has been developed and is currently being implemented.

Acknowledgments

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