Report of the Australian National Polio Reference Laboratory, 1 July to 31 December 2000

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Abstract

The Australian National Polio Reference Laboratory at the Victorian Infectious Diseases Reference Laboratory (VIDRL) is responsible for processing and testing samples for poliovirus from all Australian patients with acute flaccid paralysis and for identifying and characterising polioviruses recovered from untyped enteroviruses submitted from Australian laboratories. From 1 July to 31 December 2000, a total of 12 specimens from 7 patients with AFP were referred to the NPRL. Poliovirus type 3 Sabin-like was isolated from samples from 2 patients with suspected vaccine- associated paralytic poliomyelitis. No viruses were isolated from samples from the remaining 5 patients. Since 1995 a total of 1325 isolates have been referred for testing from laboratories throughout Australia. Seven hundred (53%) were confirmed as Sabin vaccine-like polioviruses, 542 (41%) were non-polio enteroviruses and 82 (6%) yielded no virus or viruses other than enteroviruses. At Kyoto, Japan in October 2000, the Western Pacific Region of the World Health Organization was declared wild polio-free. This represents a significant step towards the global eradication of poliovirus with one quarter of the world's population free of endemic infections from wild poliovirus. Surveillance of AFP and containment of wild polioviruses has been coordinated at the VIDRL. Since February 2000, Australia has been developing and implementing a plan for the containment of wild poliovirus stocks and potentially infectious materials. *Commun Dis Intell* 2001;25:54-58.

Keywords: poliomyelitis, acute flaccid paralysis, enteroviruses, polio certification, VAPP

Introduction

Poliomyelitis is a nationally notifiable disease and should be reported by all States and Territories to the National Notifiable Diseases Surveillance System.[1] Enterovirus surveillance of acute flaccid paralysis (AFP) cases is important in confirming that cases are not polio.

In 1988, the World Health Organization (WHO) made a commitment to the global eradication of poliomyelitis by the year 2000. An international laboratory network[2] has been progressively established and now consists of a three-tiered system incorporating specialised, regional and national or sub-national laboratories.

The Commonwealth health department supported the establishment of the Australian National Polio Reference Laboratory (NPRL) at the Victorian Infectious Diseases Reference Laboratory (VIDRL) in 1994. The laboratory has served as a regional reference laboratory for the Western Pacific Region (WPR) of WHO since 1990. Surveillance of AFP was initiated in March 1995 and is currently coordinated at the VIDRL in collaboration with the Australian Paediatric Surveillance Unit (APSU) and the NPRL. AFP surveillance is a highly sensitive monitor to ensure that every possible case of paralytic poliomyelitis is detected, investigated and characterised.[3] Based on figures of other non-endemic countries, Australia should be detecting at least one case of AFP per 100,000 children below the age of 15 years each year.

The WHO Western Pacific Region, of which Australia is a member nation, was declared free of wild poliovirus in October 2000. Although significant progress has been made to eradicate wild poliovirus worldwide, there are some countries remaining that have yet to reach this target. For this reason, high quality surveillance of AFP needs to be maintained as part of an ongoing process for the detection of wild poliovirus importations.

Whilst Sabin oral polio vaccine (OPV) remains part of the Australian Standard Vaccination Schedule,[4] incidental polioviruses will continue to be isolated. Until global certification for poliovirus is reached, even countries like Australia that have been certified wild-poliovirus free, remain susceptible to importation. An additional mechanism to detect wild poliovirus in the community is screening all poliovirus and untyped enterovirus isolates nationwide, regardless of the source of the original sample. Further virological studies are performed in order to identify all polioviruses and subsequently characterise them as Sabin or wild-type.

This report describes the functions of the NPRL and its activities during the second half of 2000. Earlier reports of the laboratory's activities have been published.[5,6,7,8] The report of the activities of the laboratory from January to December 1998[5] includes its terms of reference and their implementation.

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Methods

Samples from AFP patients

As part of the WHO poliovirus-free certification process, every case of AFP in children under 15 years in Australia must be investigated for clinical and laboratory information and followed-up for residual paralysis at 60 days to ensure that the AFP case was not poliomyelitis.[9] Paediatricians are requested to promptly report all cases of AFP to the APSU and the AFP surveillance coordinator. Arrangements are then made for 2 stool samples to be collected 24 to 48 hours apart, within 14 days of onset of paralysis. The samples are kept at 4-8^{o}C and transported to the NPRL within 3 days of collection. If transportation of samples to the NPRL is delayed, samples must be frozen at -20^{o}C and shipped frozen when suitable arrangements can be made.

Referred specimens from non-AFP patients

Samples for non-AFP patients can be referred to NPRL to exclude poliomyelitis. Five faecal samples from 3 patients with non-AFP were transported to VIDRL for testing.

Characterisation of referred entero/polioviruses

Since 1995 the various state virology laboratories have supported the VIDRL in its task to characterise all polioviruses isolated from non-AFP patients to ensure they are Sabin vaccine-like. Incidental poliovirus islolates recovered from referred entero and poliovirus isolates are characterised by nucleic acid probe hybridisation (NAPH).

Characterisation of polioviruses

A comprehensive network of laboratories for enterovirus surveillance supplements the AFP surveillance system. All polio and untyped enterovirus isolates from Australian laboratories should be referred to the NPRL for identification and intratypic differentiation. The staff of the Virology and Serology Laboratory Reporting Scheme send reminders to all laboratories reporting polio and untyped enteroviruses to refer these strains to the NPRL. This scheme ensures that no wild polioviruses should go undetected within the Australian community.

Results

Samples from AFP patients

During the second half of 2000, a total of 12 stool samples from 7 patients with AFP were referred to the NPRL (Table 1). Two patients were from Queensland and New South Wales and one each from Victoria, South Australia and the Northern Territory.

Onset dates were available for 6 patients, of whom 5 had stool samples collected within 14 days. Six samples were transported to VIDRL within 3 days of collection.

Faecal samples from 5 patients failed to yield any viruses. Sabin-like poliovirus type 3 was isolated from faecal samples from 2 patients with suspected vaccine-associated paralytic poliomyelitis (VAPP).

One 5-month-old boy from Queensland who had received a second dose of Sabin OPV 18 days before the onset of encephalomyelitis and AFP (Table 1, Case 1), had 2 stool samples collected 5 and 7 days after onset of paralysis. The referring hospital reported the detection of enteroviral ribonucleic acid by polymerase chain reaction (PCR). The NPRL identified poliovirus type 3 from the first faecal sample. The isolate was characterised as poliovirus type 3 (Sabin) by NAPH and enzyme immunoassay (EIA). The second sample failed to yield a virus. Testing of paired sera collected 2 and 20 days after onset of paralysis suggested the patient had antibodies to all 3 poliovirus strains but no significant rise in antibody titres was observed. Both faecal samples were referred to the Women's and Children's Hospital in Adelaide. Culture results confirmed *Clostridium botulinum* type A. Assay for toxin detection could not be performed due to insufficient residual volume of the 2 samples. The case was considered as non-polio by the Polio Expert Committee[9] and subsequently classified clinically as infant botulism due to the patient's improved condition.

Table 1. Results of specimens tested for enteroviruses from patients with AFP, Australia, 1 July to 31 December 2000

State/ Territory	Specimen date (stool)	Result	Paralysis onset date	Date of last OPV	Serum collection date	Serum titre result		
						P1	P2	P3
Case 1 (Qld)	6 Sep 2000	P3 Sabin	1 Sep 2000	14 Aug 2000	3 Sep 2000	256	>724	181
	8 Sep 2000	NV14			21 Sep 2000	228	>724	144
Case 2 (Qld)	7 Sep 2000	NV14	1 Sep 2000	>3 weeks				
	8 Sep 2000	NV14						
Case 3 (Vic)	19 Sep 2000	P3 Sabin	14 Sep 2000	7 Sep 2000	19 Sep 2000	>724	>724	81
Case 4 (SA)	15 Oct 2000	NV14	8 Oct 2000	>3 weeks				
	17 Oct 2000	NV14						
Case 5 (NSW)	23 Oct 2000	NV14	3 Oct 2000	>3 weeks				
	24 Oct 2000	NV14						
Case 6 (NT)	29 Oct 2000	NV14	23 Oct 2000	16 Oct 2000				
Case 7(NSW)	8 Dec 2000	NV14	INP	INP				
	12 Dec 2000	NV14						

NV14.No virus isolated after 14 days incubation in cell culture.

INP. Information not provided.

One 5-month-old female from Victoria (Case 3) with decreased movement and poor attachment to the breast had received OPV 7 days earlier. The stool and serum samples collected from the patient 5 days after onset of paralysis were referred to the VIDRL. Initial testing identified an enterovirus by PCR. The poliovirus type 3 strain isolated from the patient's stool sample was characterised as Sabin-like using NAPH and EIA. Neutralisation tests performed on the serum sample suggested that the patient had antibodies to all 3 poliovirus strains (Table 1). Further investigation concluded infant botulism as a final diagnosis. A faecal sample sent to the Women's and Children's Hospital in Adelaide yielded a *Clostridium botulinum* type B toxin. The organism could not be cultured from the faecal sample.

Referred specimens from non-AFP patients

Of 2 patients from Queensland 1 had calf muscle pain following OPV and another was diagnosed with symmetrical polyneuropathy. No viruses were isolated from these samples.

The patient from the Northern Territory was diagnosed with viral meningitis. The referring laboratory cultured and identified poliovirus type 1 from the patient's cerebrospinal fluid but considered it to be a laboratory contaminant. The isolate was referred to the NPRL and tested positive for Poliovirus type 1 by PCR and Poliovirus type 1 Sabin vaccine-like by NAPH. Faecal samples collected 2 months later and referred to the NPRL failed to yield any viruses. A neutralisation test to detect poliovirus antibodies was performed on paired serum samples. The first and second serum samples were collected 2 days and almost 2 months respectively after the collection of the cerebrospinal fluid. Although the patient had poliovirus antibodies to all 3 serotypes, no significant rise in titres was observed.

Characterisation of referred entero/polioviruses

During July to December 2000, a total of 121 polio and enterovirus isolates from 4 states were referred to the NPRL for testing. Ninety-six (79%) were referred from Western Australia, 21 (17%) from Victoria, 2 (2%) from Tasmania and 2 (2%) from South Australia. Of these 121 isolates, 34 (28%) were characterised as Sabin vaccine-like polioviruses. Forty-four (37%) were non-polio enteroviruses, 42 (35%) could not be recovered and one (1%) isolate was identified as adenovirus type 2.

Between 1995 and December 2000, a total of 1325 isolates from within Australia have been tested at the VIDRL. Table 2 summarises the cumulative results of testing of these isolates during this period.

Discussion

Surveillance and investigation of acute flaccid paralysis

Surveillance has played an important role in certification of Australia and the other countries of the WHO Western Pacific Region as being wild poliovirus-free. As part of the polio eradication process, it is important to investigate not only the polio cases but also conditions that may resemble polio clinically.[10] The main causes of AFP in Australia in the last 5 years have been Guillain Barré Syndrome and transverse myelitis.[9] However, 2 infants recently presented with AFP following immunisation with oral polio vaccine (OPV). Poliovirus type 3 (vaccine-like) was isolated from the faecal samples of both patients, which suggested possible cases of VAPP. Further testing of the faecal samples concluded infant botulism was the cause of both patients' paralysis. Exclusion of VAPP as the differential diagnosis was made possible due to adequate faecal sample collection and extensive laboratory testing. The NPRL plans to sequence all poliovirus isolates from AFP cases and is currently performing molecular characterisation of the poliovirus type 3 isolates from the suspected VAPP cases to determine whether these viruses are true Sabin OPV or vaccine derived.

Surveillance of AFP is considered a highly sensitive indicator of wild poliovirus activity since nearly all cases of paralytic polio have AFP.[10] Based on figures in non-endemic countries, Australia should identify 40 cases of AFP in children below the age of 15 years annually,[11] or 20 cases for this reporting period.

Despite the continuous publicity and information provided to highlight the critical role of AFP surveillance, the expected number of AFP cases investigated was not reached in this reporting period. Between January to December 2000, samples from a total of 20 AFP cases (13 cases from an earlier report[8]) were referred to the NPRL. Until December 1999, there had been an increasing trend in the number of AFP cases reported, per calendar year – samples from 27 cases were referred to the NPRL in 1999. However, a decrease in the number of referred samples in 2000 may be attributed to complacency due to Australia approaching wild poliovirus-free certification. Also worth noting is the failure of hospitals to refer faecal samples collected from 4 AFP cases to the NPRL during the reported period. Although notification to the APSU had been made, these samples were tested in other laboratories for various pathogens. Samples from only one of these cases were tested for virus culture. A further 9 notifications to the APSU were made but specimens were not collected for laboratory investigation. If all notified AFP cases – 20 in total – had specimens collected and referred to the NPRL, Australia would have met the WHO criteria for AFP surveillance for this reporting period. Continuing commitment from paediatricians to report all cases of AFP, return study questionnaires and arrange collection and transportation of faecal samples in accordance with the WHO protocol to the NPRL is required. The Department of Health and Aged Care is committed to continuing AFP surveillance at an acceptable level until global certification of poliovirus-free status is achieved.

Western Pacific Regional Certification

The Regional Commission for the Certification of Poliomyelitis Eradication in the WPR was established in 1996. Its task has been to set the standards and criteria to be followed by every country and area in the Region.[10] The Regional Certification Commission met in Kyoto, Japan, during October 2000 to review each country's final documentation for certification. Every country of the WPR including Australia, provided evidence consistent with the absence of indigenous wild poliovirus for a minimum of 3 years whilst undertaking high quality surveillance. Other criteria to be met were the validation of the certification documentation by a National Certification Committee of each country and development of plans of action to detect and respond to importation of wild poliovirus and to contain wild poliovirus and potentially infectious materials. Once the Regional Certification Commission was satisfied with the

Table 2. Cumulative summary of identification of enteroviruses and intratypic differentiation of polioviruses from Australian laboratories from 1995 to December 2000 performed at the NPRL (percentage of total in brackets)

State	Year	Polio Sabin-like	Non-polio enterovirus	Non-enterovirus/ negative	Total
Victoria	1995	9			9
	1996	17			17
	1997	5			5
	1998	7			7
	1999	19			19
	2000	24			24
Queensland	1995	41	5	8	54
	1996	99	4	9	112
	1997	41			41
	1998	8	15	2	25
	1999	2			2
	2000				
Western Australia	1995/6	133	384	5	522
	1997	32	76		108
	1998			2	2
	1999	3	9	9	21
	2000	13	44*	47†	104
Tasmania	1995	1			1
	1996	3			3
	1997	4			4
	1998	4			4
	1999	4			4
	2000	2			2
New South Wales	1994	5			5
	1995	76	5		81
	1996	35			35
	1997	39			39
	1998	30			31‡
	1999	31			31
	2000	4			4
South Australia	1997	3			3
	1998	3			3
	1999	1			1
	2000	2			2
Total	1995-2000	700 (53%)	542 (41%)	82 (6%)	1,325

* Two non-poliovirus isolates were cultured in L20B and RD cells.

† Includes one adenovirus type 2 isolate.

‡ Includes one non-Sabin poliovirus type 2.

documentation for each country in the Region, a decision for certification was reached. At the Kyoto Meeting for the Declaration of Poliomyelitis Eradication in the WPR on 29 October 2000, the Regional Director of the WPR WHO declared that the Region had achieved polio-free status.

Maintaining poliovirus-free status

The WPR is now the second region in the world to have achieved a wild poliovirus-free status. The Americas Region was certified wild polio-free in 1994. However, wild poliovirus is still circulating in countries throughout other non-certified regions. The threat of importation exists in Australia due to immigration from endemic countries. To prevent the reintroduction of wild poliovirus, it is imperative that we maintain high vaccination coverage and effective surveillance of AFP cases. The one case of poliomyelitis resulting from the importation of wild poliovirus from India into China in 1999[12] highlights the importance of high quality AFP surveillance and a national strategy to respond to importations.

An outbreak of poliomyelitis in the Caribbean between July and November 2000 was caused by a mutant form of a type 1 poliovirus from OPV.[13] The reverted strain had

characteristics of both neurovirulence and transmissibility, which the live attenuated strains in OPV do not possess. In Egypt, cases of poliomyelitis between 1988 and 1993 were associated with vaccine-derived poliovirus type 2.[14] In both areas, circulation of vaccine-derived polioviruses occurred in areas with low OPV coverage.

These examples highlight the need for Australia to remain vigilant by maintaining adequate AFP surveillance, high immunisation coverage, a plan to effectively respond to a wild-type poliovirus importation and ensuring adequate laboratory containment of wild poliovirus materials[15] until global certification has been achieved.

Abbreviations

National Polio Reference Laboratory (NPRL), Victorian Infectious Diseases Reference Laboratory (VIDRL), World Health Organization (WHO), Western Pacific Region (WPR), Australian Paediatric Surveillance Unit (APSU), Vaccine-Associated Paralytic Poliomyelitis (VAPP), Acute Flaccid Paralysis (AFP), Oral Polio Vaccine (OPV), Polymerase Chain Reaction (PCR), Nucleic Acid Probe Hybridisation (NAPH), Enzyme Immunoassay (EIA)

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Addendum

Stambos V, Brussen KA, Turnbull A et al. Report of the National Polio Reference Laboratory: 1 January to 30 June 2000. *Commun Dis Intell:* 2000;24:300-303.

The last reported case of indigenous wild poliovirus in the Western Pacific Region was in Cambodia in March 1997 and not in 1977 as reported in the discussion section. The authors apologise for any inconvenience that this may have caused.