Annual report of the Australian National Poliovirus Reference Laboratory, 2003

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Abstract

The Australian National Poliovirus Reference Laboratory was established in late 1994, as part of Australia's commitment to the World Health Organization's (WHO) polio eradication program. The laboratory continues to play a pivotal role in maintaining Australia's polio-free status through surveillance for cases of acute flaccid paralysis (AFP), the main clinical presentation of poliomyelitis, and the testing of specimens from these cases. The annual notification rate for eligible cases of AFP in Australia for 2003 was 0.83 per 100,000 children less than 15 years of age. The annual non-polio AFP rate after classification of cases by the polio expert committee was 0.68 per 100,000, 32 per cent below WHO's annual target. While no polioviruses were isolated from the specimens tested from the 27 cases of AFP in 2003, a novel enterovirus (enterovirus 75) was isolated from one case and enterovirus 71 was isolated from another. During the same period 12 polioviruses, referred from cases other than AFP, tested as Sabin-like by the WHO approved methods of intratypic differentiation. The importation of wild polioviruses from endemic Nigeria into surrounding countries of Africa during 2003, highlights the importance of the continuation of AFP surveillance and high quality laboratory activities throughout the world until global eradication of polio is certified. *Commun Dis Intell* 2004;28:339–344.

Keywords: poliovirus, acute flaccid paralysis, surveillance, enterovirus

Introduction

In May 1988, the World Health Assembly adopted a resolution for the global eradication of poliomyelitis. The World Health Organization (WHO) implemented a program to achieve this goal through high levels of polio vaccination coverage, predominantly with the Sabin oral polio vaccine (OPV). In addition, the program initiated surveillance for cases of acute flaccid paralysis (AFP), the most commonly observed clinical manifestation of poliovirus infection, and the establishment of a global laboratory network accredited for the testing of specimens from AFP cases.[1]

The Australian National Polio Reference Laboratory (ANPRL) was established in late 1994 at the Victorian Infectious Diseases Reference Laboratory (VIDRL), as part of Australia's commitment to the WHO polio eradication program. The laboratory also serves as the national polio laboratory for the Pacific Island countries and as a regional polio reference laboratory for the WHO Western Pacific Region. Surveillance for AFP in Australia was initiated in March 1995 by the then Federal Government Department of Human Services and Health. The surveillance program has been co-ordinated at VIDRL since 2000, in collaboration with the Australian Paediatric Surveillance Unit (APSU).

Poliomyelitis is a notifiable disease in all states of Australia while AFP is also a notifiable disease in Queensland.[2] In a country that is not endemic for polio, such as Australia, the WHO indicator target for AFP cases for children aged less than 15 years, is one case per 100,000 population. For Australia, this represents 40 AFP cases per year. The WHO target for laboratory testing is that at least 80 per cent of notified AFP cases have adequate stool specimens collected (two specimens at least 24 hours apart and within 14 days of onset of paralysis) and tested in a WHO accredited laboratory.

Australia's childhood immunisation schedule includes four doses of the live, attenuated OPV. Doses are recommended at 2, 4 and 6 months of age with a booster prior to school entry.[3] OPV is a trivalent vaccine comprising all three poliovirus serotypes. After

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administration of the vaccine, the viruses multiply in the gut of the recipient and can be excreted for up to six weeks from immunocompetent individuals.[4] Longer excretion from immunocompromised recipients has been documented.[5,6] Therefore, poliovirus can be isolated from stool specimens from individuals with clinical symptoms other than AFP during routine laboratory testing. These viruses should be subjected to further testing to confirm their vaccine origin and hence considered an incidental finding. A further boost with polio vaccine is recommended for people travelling in the remaining polio endemic regions of Africa, the Eastern Mediterranean and South Asia.[3]

The activities of the ANPRL in 2003 are summarised in this annual report which includes a comparison of AFP surveillance in Australia against the major targets nominated by WHO.

Methods

AFP surveillance is co-ordinated by the ANPRL in collaboration with the APSU. Briefly, any doctor in Australia seeing a patient under 15 years of age presenting with AFP or a person of any age suspected of an acute poliomyelitis infection is requested to telephone the AFP co-ordinator at VIDRL to notify the case. The doctor is requested to collect two stool specimens, 24 hours apart and within 14 days of onset of paralysis, and forward them to the ANPRL for testing. Paediatricians also notify cases of AFP through a monthly reporting scheme to the APSU. The clinicians who notify a case of AFP are requested to complete a questionnaire, which is reviewed, in conjunction with laboratory results, by the Polio Expert Committee (PEC). Cases are classified by the committee as either (i) non-polio AFP, (ii) poliomyelitis due to wild poliovirus, vaccine-derived poliovirus (VDPV) or vaccine-associated paralytic poliomyelitis, or (iii) non-AFP.

Laboratory methods were described in detail in the 2001 ANPRL annual report.[7] In brief, stool specimens received from AFP cases are extracted with a 10% v/v chloroform solution and inoculated into WHO approved cell lines. This includes the L20B cell line, a genetically modified mouse cell line that expresses the human poliovirus receptor. All polioviruses, whether from AFP cases or other sources, are tested by enzyme-linked immunosorbent assay (ELISA) and genetic methods, (PCR), for the differentiation between wild and the OPV strains of poliovirus. The poliovirus ELISA is sensitive to antigenic drift due to mutations accumulated during viral replication. The VP1 gene is sequenced for any poliovirus with discordant results by the two methods of intratypic differentiation; that is, Sabin-like by PCR but non-Sabin-like or double reactive (equal avidity with both Sabin and non-Sabin antiserum) by ELISA. The poliovirus is reported as (i) a wild type poliovirus, (ii) Sabin vaccine-like or (iii) a vaccine derived poliovirus (VDPV) if there is more than one per cent variation in the VP1 gene sequence compared to the parental Sabin strain.

Results

Acute flaccid paralysis surveillance

According to the WHO criteria, eligible cases are patients who are Australian residents and aged less than 15 years on the date of paralysis onset. However, the PEC will review cases of suspected poliomyelitis in people of any age. Forty-four notifications of AFP in Australia were received in 2003. Duplicate notifications were received for eight cases. Thirty-three cases of AFP were from patients less than 15 years of age and three cases were patients 15 years or older. Of the 33 eligible cases, the PEC classified 27 as non-polio AFP, two cases are pending review and classification and a further four cases require more information from the referring doctor before a final classification can be made. Thus, the annual AFP notification rate for eligible cases in Australia in 2003 was 0.83 per 100,000 children less than 15 years of age, 17 per cent below the WHO target of 1.0 per 100,000 (Table 1). The annual non-polio AFP rate in 2003, after classification of cases by the PEC, was 0.68 per 100,000 population, 32 per cent below the WHO target. Twelve of the 27 cases classified as non-polio AFP by the PEC were diagnosed as Guillian-Barré Syndrome.

Laboratory testing of specimens from acute flaccid paralysis cases

The ANPRL tested 35 stool specimens and one virus isolate from 19 cases of AFP in children aged less than 15 years, in 2003. This included one case with onset of symptoms in late 2002. A further two stool specimens were referred from one case of AFP in a person greater than 15 years. The WHO criteria for adequate stool sampling of AFP cases is two specimens collected at least 24 hours apart and within 14 days of onset of paralysis in at least 80 per cent of eligible cases. During 2003, 24 per cent of eligible AFP cases had adequate stool specimens (Table 1). No polioviruses were isolated from any specimens tested from AFP cases during 2003 (Table 2).

Enterovirus 71 was isolated from two stools from one AFP case. A portion of the VP1 gene was sequenced and a search of the GenBank database with the BLAST software indicated the greatest homology to strains of genogroup C1 isolated in Malaysia in the late 1990s.

Table 1. Acute flaccid paralysis surveillance in Australia, compared with World Health Organization indicator targets for children less than 15 years, 2003

WHO* indicator target for AFP† cases of children less than 15 years	Australia's surveillance for AFP cases with onset in 2003	Australia's AFP surveillance rates for 2003
Non-polio AFP case rate of 1 per 100,000 population (40 cases for Australia in 2003)	33 cases of AFP notified	AFP notification rate: 0.83 per 100,000 population
	27 cases classified by the PEC as non-polio AFP‡	Non-polio AFP case rate: 0.68 per 100,000 population
More than 80% of notified AFP cases with 2 adequate stool specimens collected at least 24 hours apart within 14 days of onset of paralysis	8 AFP cases with 2 or more specimens per case	Referral of adequate specimens from AFP cases: 24% of case notifications (8/33)

* WHO World Health Organization.

† AFP acute flaccid paralysis.

‡ A further two cases are pending review by the Polio Expert Committee (PEC) and four cases require further information from the referring doctor before final classification.

Table 2. Testing of specimens and isolates referred to the Australian National Poliovirus Reference Laboratory, 2003

Result	Results from acute flaccid paralysis cases		Isolations from referred samples	Total
	< 15 years	≥ 15 years		
Poliovirus Sabin-like type 1	–	–	3	3
Poliovirus Sabin-like type 1 & 2	–	–	2	2
Poliovirus Sabin-like type 1 & 3	–	–	1	1
Poliovirus Sabin-like type 2	–	–	3	3
Non-polio enterovirus *	4	–	11	15
No virus isolated	32	2	13†	47
Total	36	2	33	71

* Enterovirus type 71 was isolated from two stool specimens of one AFP case and enterovirus type 75 was isolated from a stool specimen and an isolate of another case. Testing of the referred samples identified two echovirus type 25, two each of coxsackievirus types B3 and B4 and single isolations of coxsackievirus types B2 and B5. The specific identification of three isolates is pending.

† Includes specimens tested for poliovirus as part of a differential diagnosis and also for ongoing shedding from a recently immunised patient with an immune deficiency. No virus may have been isolated from the remaining referred isolates due to loss of titre in transit and/or not passaging between different cell lines.

A non-polio enterovirus was identified from the stool of another case of AFP. A comparison of the VP1 gene sequence to the GenBank database determined no significant homology to the known enterovirus prototypes. The sequence was referred to collaborators at the Centers for Disease Control and Prevention, United States of America, who reported the virus to be enterovirus type 75, a newly described enterovirus. (Dr M Steven Oberste, Research Microbiologist, Respiratory and Enteric Viruses Branch, Centres for Disease Control and Prevention, Atlanta, United States of America, personal communication).

Specimens and isolates referred for isolation and identification

Thirty-three specimens and isolates were received by the ANPRL from sources other than AFP during 2003. Six poliovirus type 1, five poliovirus type 2 and one type 3 poliovirus were isolated from nine samples (Table 2). A mixture of two different serotypes was identified from three of the samples. All 12 polioviruses isolated tested as Sabin vaccine-like by the WHO approved methods for intratypic differentiation.

Amongst the referred samples from sources other than AFP cases (Table 2), three stool specimens were from a child immunised with OPV, who was subsequently diagnosed with a T-cell deficiency. The specimens were tested to determine if ongoing shedding of the OPV virus strains was occurring, but no virus was isolated. Poliomyelitis was considered as part of the differential diagnosis of another child with ataxia. Two stool specimens were tested but no virus was isolated from either specimen.

Laboratories within Australia are encouraged to forward their untyped enteroviruses to ANPRL for identification. If they are confirmed as poliovirus they are tested by the WHO approved methods of intratypic differentiation to characterise the isolate. Of the 33 specimens and isolates tested, 18 were untyped enteroviruses from a laboratory in South Australia. Five polioviruses were identified amongst these viruses and all tested as Sabin vaccine-like.

Eight non-polio enteroviruses were identified in 2003, originating from Victoria, South Australia and Queensland. (Table 2). Two echovirus type 25, two coxsackievirus B3, two coxsackievirus B4 and single isolates of coxsackievirus B2 and B5 were identified by sequence homology by comparison of a portion of the VP1 gene to the GenBank database and the serotype was confirmed with monospecific antiserum. A further three non-poliovirus enteroviruses are yet to be identified.

A summary of enterovirus testing of specimens and isolates referred to the ANPRL from within Australia between 1995 and 2003 are presented in Table 3.

Poliovirus serology

Serum specimens were referred for poliovirus serology from two patients. One patient had an encephalomyelitis of unknown aetiology while the second patient had paraplegia. Results from both patients were inconclusive as no acute serum was available for either patient.

Regional reference laboratory activities

Three hundred and fifty specimens and isolates were referred to the ANPRL from countries of the Western Pacific Region in the laboratory's role as a WHO regional reference laboratory. As part of an ongoing laboratory quality assurance program, the laboratory received 146 specimens and isolates from the National Poliovirus Laboratory of Papua New Guinea and 120 from the National Laboratory of Viet Nam, Ho Chi Minh City. In additional roles as a National Polio Reference Laboratory, four specimens were received from two cases of AFP in Brunei Darussalam and 22 specimens from 11 cases of AFP were received from the Pacific Island countries. Echovirus type 6 was isolated from two cases of AFP in Fiji. Specimens and isolates were also referred from Malaysia, Mongolia, and the Philippines for further identification and characterisation.

A poliovirus type 1 isolated during a survey of healthy children to supplement AFP surveillance in Mongolia, was referred to ANPRL. The intratypic differentiation test result was discordant: Sabin-like by PCR and non-Sabin-like by ELISA. The complete VP1 gene sequence was confirmed by the WHO Global Specialized Laboratory at the National Institute of

Table 3. Summary of enterovirus testing at the Australian National Poliovirus Reference Laboratory, 1995 to 2003

Year	Poliovirus		Non-polio enterovirus	Non-enterovirus detected or no virus detected	Total samples tested
	Sabin-like	Non-Sabin-like*			
1995	190		200	13	403
1996	224		198	9	431
1997	124		76	0	200
1998	52		15	4	71
1999*	60	1	9	9	79
2000	45		44	47	136
2001*	46	5	33	75	159
2002†	36		21	49	106
2003	9		15	47	71

* Untyped enterovirus or uncharacterised poliovirus isolates were referred for further testing after completion of a laboratory inventory. Some of the isolates tested as non-Sabin-like and were subsequently identified as wild type poliovirus prototype strains and were destroyed.

† Two poliovirus isolates from non-AFP sources had discordant results by intra-typic differentiation. Sequencing confirmed the isolates as Sabin-like, with <1.0 per cent variation from the parental Sabin strain.

infectious Disease, Tokyo, to have 98.7 per cent homology to Sabin type 1 prototype sequence, thus classifying the virus as a VDPV (greater than 1% variation from Sabin VP1 prototype sequence). The child was later determined to have a previously undiagnosed immune deficiency and extensive surveillance activities found no evidence of circulation of the VDPV within the population.

Discussion

Australia has continued to struggle to meet the WHO standard criteria for AFP surveillance in a non-polio endemic country, of detecting at least one case of AFP per 100,000 children aged less than 15 years. Since the introduction of AFP surveillance in 1995, the target was met only in 2000 (1.15) and 2001 (1.13).[7] However, this dropped to 0.75 cases per 100,000 population less than 15 years of age in 2002[8] and to 0.68 in 2003. The rate of stool sampling in 2003 was 24 per cent, well below the WHO target of 80 per cent of AFP cases in children less than 15 years. When considering the notifications involving patients of all ages, 17 of the 36 AFP cases (47%) had at least one stool specimen collected in 2003. The 2003 rate for specimen collection was similar to that reported between 1995 and 1999 and in 2002,[7,8] while stool collection rates rose to 31 per cent and 36 per cent in 2000 and 2001, respectively.

A capture-recapture study of AFP cases determined that the incidence of AFP in Victoria was consistently under reported.[9] A more recent study was undertaken with a grant from the Australian Government Department of Health and Ageing to investigate differences in AFP notification rates by state. The study evaluated the disparity in the AFP notification rates from one state to another and concluded that the engagement of the state based departments of health to promote and monitor AFP as a public health issue, may assist in increasing Australia's AFP notification rate.[10]

The two poliovirus serology results in this report highlight the need for adequate sampling (acute and convalescent serum) for a definitive conclusion to be made from the polio antibody test. The test cannot differentiate between an immune response from recent exposure to wild poliovirus, vaccine-derived poliovirus or OPV. If an acute polio infection is suspected, stool specimens remain the specimen of choice.

The development of molecular techniques for the identification of enteroviurses has led to the description of previously untypeable enteroviruses. As yet, no antisera are available to these new enteroviruses and their identification is based on genotyping rather than the traditional method of serotyping.[11,12] The identification of enterovirus type 75 from an Australian AFP case was based on genotyping of the virus and represents, to our knowledge, the first documentation of this virus in Australia. Enterovirus type 75 has been isolated from patients with a variety of illnesses including AFP.[13]

The increase in severity of disease and outbreaks associated with enterovirus type 71 in recent years within the Asia-Pacific region, suggests the need for the specific identification and monitoring of enterovirus isolations within Australia.[14,15] The enterovirus type 71 isolated from one case of AFP in Australia in 2003, was closely related to viruses of genogroup C1 detected in the Malaysian peninsular during 1997–2000.[14] Enterovirus type 71 of genogroup C1 was determined to be responsible for the outbreak of hand, foot and mouth disease in Malaysia in 2000, and has been suggested to have the potential to cause future epidemics within the Asia-Pacific region.[15]

The number of untyped enteroviruses referred from within Australia to the ANPRL for further identification and characterisation has dwindled since 1995. The increasing use of PCR directly on extracted specimens in many laboratories has possibly decreased the number of virus isolates available for further testing. The isolation of enterovirus types 71 and 75 described in this report and the detection of polioviruses with discordant intratypic differentiation results from sources other than AFP in 2002,[8] exemplify the need for the specific identification of enteroviruses.[16]

During 2003, of the 784 wild polioviruses isolated globally, 51 were detected in eight African countries previously considered polio-free.[17] Immunisation coverage against polio is believed to have dropped recently in these countries.[17,18] The polioviruses were determined to have been imported from one of the largest remaining areas endemic for polio that spans northern Nigeria and southern Niger. For Australia to retain its polio-free status, it must maintain a high level of polio vaccination coverage until global eradication has been certified, as well as supporting the continuation of surveillance for AFP cases and laboratory testing of specimens.

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