

ANNUAL REPORT OF THE AUSTRALIAN NATIONAL POLIOVIRUS REFERENCE LABORATORY 2009

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Abstract

The Australian National Poliovirus Reference Laboratory (NPRL) is accredited by the World Health Organization (WHO) for the testing of faecal specimens from acute flaccid paralysis (AFP) cases and operates as a regional poliovirus reference laboratory for the Western Pacific Region. The NPRL, in collaboration with the Australian Paediatric Surveillance Unit, co-ordinates surveillance for cases of AFP in children in Australia, according to criteria recommended by the WHO. Specimens are referred from AFP cases in children and suspected cases of poliomyelitis in persons of any age. The WHO AFP surveillance performance indicator is 1 non-polio AFP case per 100,000 children less than 15 years of age. In 2009, the Polio Expert Committee classified 48 cases as non-polio AFP, a rate of 1.17 cases per 100,000 children less than 15 years of age. An additional WHO AFP surveillance performance indicator is that more than 80% of notified AFP cases have 2 faecal samples collected 24 hours apart and within 14 days of onset of paralysis. Adequate faecal samples were received from 16 (33.3%) of the 48 classified cases. A poliovirus was referred via the Enterovirus Reference Laboratory Network of Australia from a non-AFP case and was determined to be Sabin-like. This case most likely represents an importation event, the source of which was not identified, as Australia ceased using Sabin oral polio vaccine in 2005. The last report of a wild poliovirus importation in Australia was from Pakistan in 2007. In 2009, 1,604 wild poliovirus cases were reported in 23 countries with Afghanistan, India, Nigeria and Pakistan remaining endemic for poliomyelitis. *Commun Dis Intell* 2010;34(3):277–284.

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

Introduction

The National Polio Reference Laboratory (NPRL) is responsible for the virological testing of faecal specimens from cases with a clinical suspicion of poliomyelitis. This includes cases of acute flaccid paralysis (AFP)—a major clinical presentation of poliomyelitis—in children less than 15 years of age and cases of suspected poliomyelitis in patients of

any age. The World Health Organization (WHO) recommends that 2 faecal specimens be collected from cases of AFP for virological investigation at least 24 hours apart and within 14 days of the onset of paralysis. 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 NPRL at the Victorian Infectious Diseases Reference Laboratory (VIDRL). Laboratory testing may exclude poliovirus as the causative agent of AFP. Enteroviruses other than poliovirus have been associated with AFP.

From November 2005, inactivated poliomyelitis vaccine (IPV) replaced oral poliomyelitis vaccine (OPV) in the National Immunisation Program.¹ IPV is administered to children at 2, 4 and 6 months of age, with a booster dose at 4 years of age. With the removal of OPV, containing 'live' attenuated virus, from the immunisation schedule, any poliovirus identified by Australian virology laboratories requires further investigation to determine its origin, as it represents an importation event.

It is important that Australia maintains high levels of polio vaccine coverage to avoid a resurgence of poliomyelitis in the event of a wild poliovirus importation. Reinforcement of this recommendation is evidenced by the large type 1 wild poliovirus outbreak in Tajikistan in 2010, a country with reportedly similar polio vaccination coverage (87% in 2008) and AFP surveillance performance as Australia (non-polio AFP rate of 1.4 in 2009).² As of 12 July 2010 there have been 413 confirmed cases of poliomyelitis with 19 deaths in Tajikistan.³ People travelling to polio endemic countries and countries with recent wild poliovirus importations should receive a booster polio vaccine prior to departure, or a full course of vaccination if they are unsure of their vaccination history. Individuals who are at continuing risk of infection,⁴ such as health care workers, are recommended to have a booster polio vaccine every 10 years.⁵ The WHO provides a searchable database of global case counts and surveillance data at http://apps.who.int/immunization_monitoring/en/diseases/poliomyelitis/case_count.cfm

The Australian NPRL is also the National Poliovirus Reference Laboratory for Brunei Darussalam, Papua New Guinea and the Pacific Island countries, and is a regional reference laboratory for the

WHO Western Pacific Region. Specimens and isolates are referred to the laboratory from national laboratories throughout the region in accordance with requirements determined by the WHO.

Methods

AFP surveillance was initiated by the Australian Government in 1995 in collaboration with the Australian Paediatric Surveillance Unit (APSU) as part of Australia's commitment to the WHO poliomyelitis eradication program. Since 2000, AFP surveillance has been co-ordinated by VIDRL in collaboration with the APSU.

The strategy adopted for AFP surveillance is as follows:

- Paediatricians reviewing a patient less than 15 years of age and presenting with AFP, or a clinician reviewing a patient of any age suspected of poliomyelitis, are requested to notify the NPRL (telephone 03-9342 2607, email polio@mh.org.au). Notification of the case is also included on the paediatrician's monthly report card to the APSU (<http://www.apsu.org.au/>).
- Two faecal specimens should be collected 24 to 48 hours apart and within 14 days of onset of paralysis. Collection of specimens within these time frames will enable them to be classified as adequate by WHO.
- The faecal specimens are referred free of charge for testing by the NPRL, which is accredited by WHO for this purpose.
- Upon notification of an AFP case, clinicians are forwarded a clinical questionnaire for completion.
- The Polio Expert Committee (PEC), convened by the Department of Health and Ageing (DoHA), 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 under 15 years of age with AFP (including Guillain-Barré syndrome) or an Australian of any age with paralytic illness if polio is suspected. Examples of ineligible cases are if the patient is aged 15 years or older, an overseas resident and cases notified in error or later determined to be non-AFP.
- The PEC classifies cases of AFP as:
 - poliomyelitis due to wild poliovirus, vaccine-derived poliovirus (VDPV) or vaccine associated paralytic poliomyelitis (VAPP);
 - non-polio AFP or;
 - non-AFP.
- A follow-up questionnaire is sent to notifying clinicians if the PEC requires more information regarding the AFP case before a final classification can be made.
- After each PEC meeting the Australian AFP data are forwarded to WHO for inclusion in the global AFP surveillance data published in the *Weekly Epidemiological Record* (available from <http://www.who.int/wer/en/>). Ineligible cases are not reported to WHO.
- The WHO AFP surveillance performance indicator for a polio non-endemic country is 1 non-polio AFP case per 100,000 children aged less than 15 years. For Australia in 2009, this equated to 41 cases per year, based on the Australian Bureau of Statistics data released in December 2008. An AFP surveillance scheme that satisfies the surveillance performance indicator is deemed sufficiently sensitive to detect a wild poliovirus importation in children of that country.
- 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.
- At the end of each calendar year, a number of AFP notifications remained unclassified as insufficient clinical and laboratory data were available to enable the PEC to review the cases. In 2008, after consulting with WHO, the PEC resolved to classify pending cases as 'polio compatible—zero evidence'.

Upon receipt at the NPRL, faecal specimens are treated with Minimum Essential Medium containing Hank's salts, chloroform (9.1% v/v) and foetal bovine serum (2%). The suspension is clarified and the supernatant inoculated onto a series of mammalian cell lines. Two WHO recommended cell lines are used for the isolation of poliovirus; L20B (a transgenic mouse epithelial cell line expressing the human poliovirus receptor, CD155) and RD-A (human rhabdomyosarcoma).^{6,7} The NPRL utilises 2 additional cell lines for the isolation of poliovirus and non-polio enteroviruses: BGMK (buffalo green monkey kidney) and HEL (human embryonic lung). Diagnostic laboratories in Australia are encouraged to refer enteroviruses of unknown serotype to the NPRL for further characterisation as poliovirus infection can lead to clinical presentations without paralysis such as aseptic meningitis.

A series of tests known as intratypic differentiation (ITD) are performed on poliovirus isolates to determine whether the virus is a wild poliovirus strain, OPV strain (Sabin-like) or a VDPV. In 2009, the WHO introduced diagnostic poliovirus

real time reverse transcriptase polymerase chain reaction (rRT-PCR), developed by the US Centers for Disease Control and Prevention (CDC), Atlanta, as the primary ITD method.⁸ The Australian NPRL sequences the complete poliovirus VP1 genomic region, which contains a major neutralising antibody binding site. The VP1 genomic sequence provides valuable biological information, including the number of mutations within a significant region of the OPV virus strain and it enables phylogenetic analysis of wild poliovirus to rapidly determine the likely source of the virus, as utilised in the 2007 importation.⁹

Results

Notification of acute flaccid paralysis cases

A total of 61 notifications of AFP cases were received in 2009 (Table 1). Two AFP cases were already notified by other clinicians and so were regarded as duplicate notifications.

(i) Eligible AFP cases

Sufficient information was available for the PEC to review 48 cases of AFP involving children less than 15 years of age with onset of paralysis in 2009. All cases were classified as non-polio AFP by the PEC. The 48 cases equates to a non-polio AFP rate of 1.2 cases per 100,000 children less than 15 years of age. This result meets the WHO AFP surveillance performance criterion for a polio-free country of 1 case of non-polio AFP per 100,000 children less than 15 years of age (Figure).

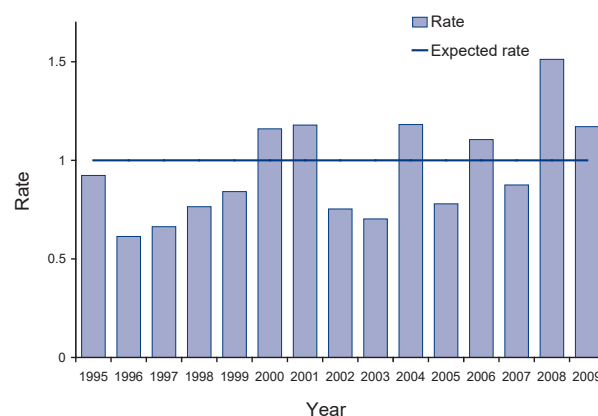
(ii) Ineligible cases

Eight cases did not meet the criteria for an eligible case. Four were later reported as non-AFP and the other four involved patients aged over 14 years; one with onset of paralysis in 2008. The cases involving patients over 14 years of age were all classified by the PEC as non-polio AFP but were not reported to the WHO since the organisation focuses on the onset of AFP in children less than 15 years of age.

Notification of acute flaccid paralysis cases by state and territory

In 2009, AFP cases were notified from all jurisdictions in Australia except for the Northern Territory (Table 2). After excluding duplicate notifications and ineligible cases, the non-polio AFP rates per

Figure: Non-polio acute flaccid paralysis rate after final classification of cases by the Polio Expert Committee



WHO AFP Surveillance Performance Indicator = 1 non-polio AFP case per 100,000 population <15 years

Table 1: Surveillance for acute flaccid paralysis (AFP) cases in children less than 15 years of age, Australia, 2009, compared with the World Health Organization (WHO) AFP surveillance performance indicators

WHO surveillance performance indicator for AFP cases in children less than 15 years*	Australia's surveillance for AFP cases in children with onset of paralysis in 2009	Australia's AFP surveillance performance in 2009
Non-polio AFP case rate of 1.0 per 100,000 children (41 cases for Australia in 2009).	60 unique cases of AFP notified 48 cases classified by the Polio Expert Committee as non-polio AFP	AFP notification rate: 1.46 per 100,000 children. Non-polio AFP case rate: 1.17 per 100,000 children.
More than 80% of classified AFP cases with 2 adequate faecal specimens collected at least 24 hours apart and within 14 days of onset of paralysis.	16 AFP cases with 2 or more adequate specimens	Referral of adequate specimens from AFP cases: 33.3% (16 of 48) of the eligible cases.

* Based on data supplied by the Australian Bureau of Statistics, estimated population, preliminary – 30 June 2008. ABS publication 3201.0.

jurisdiction exceeded the AFP surveillance performance indicator of 1 case per 100,000 children in New South Wales, Victoria, Western Australia and Tasmania. The surveillance performance indicator was not achieved in Queensland, South Australia, the Australian Capital Territory or the Northern Territory (Table 2).

Faecal collection from acute flaccid paralysis cases

WHO defines adequate specimens for poliovirus culture as 2 faecal specimens collected at least 24 hours apart and within 14 days of the onset of paralysis. A further surveillance criterion set by WHO is for adequate faecal collection from 80% of the eligible AFP cases.

In 2009, a total of 47 faecal specimens from 29 of the 48 eligible cases were tested at the NPRL (Table 1):

- 16 (33%) of the eligible cases had adequate specimens with 2 specimens collected within 14 days of symptom onset;
- 11 (23%) cases had 1 specimen collected within 14 days of onset; two of the cases had a 2nd specimen collected after 14 days;
- 2 (4%) cases had 1 faecal specimen collected more than 14 days after onset;
- no faecal specimens were received from the remaining 19 (40%) eligible cases.

The 33% (16 of 48 cases) proportion of eligible cases with adequate faecal specimen collection compares with the WHO criterion of 80%. Queensland was the only jurisdiction to reach the WHO performance indicator within the reporting period (Table 2).

Laboratory testing of specimens

Acute flaccid paralysis cases

Between 1 January and 31 December 2009, a total of 54 specimens were referred from 31 cases of AFP involving patients aged less than 15 years (Table 3). No poliovirus was isolated from any of these specimens.

A non-polio enterovirus (NPEV) was isolated from a faecal specimen of 1 patient with onset of paralysis in Victoria in February 2009. Ribonucleic acid (RNA) was extracted from the virus isolate and a fragment of the VP1 genomic region

Table 2: Notification of acute flaccid paralysis (AFP) cases, Australia, 2009, by state or territory

State or territory	Estimated population aged <15 years*	Expected number of AFP cases in 2009	Total number of notifications	Ineligible notifications	Duplicate notifications	Cases with insufficient information to classify	Eligible cases with final classification by PEC	Notification rate per 100,000 children†	Non-polio AFP rate per 100,000 children
ACT	63,874	1	1	0	0	1	0	1.0	0.0
NSW	1,332,066	13	20	2	1	0	17	1.5	1.3
NT	52,253	1	0	0	0	0	0	0.0	0.0
Qld	861,002	8	8	1	3	1	3	1.0	0.4
SA	289,309	3	2	1	0	0	1	0.7	0.3
Tas	97,012	1	1	0	0	0	1	1.0	1.0
Vic	995,096	10	25	3	0	0	22	2.5	2.2
WA	426,476	4	4	0	0	0	4	1.0	1.0
Aust	4,117,612	41	61	7	4	2	48	1.5	1.2

* Australian Bureau of Statistics, estimated population, preliminary – 30 June 2008. Australian Bureau of Statistics publication 3201.0.

† Excludes duplicate notifications and ineligible cases.

PEC Polio Expert Committee

Table 3: Test results of specimens referred to the Australian National Polio Reference Laboratory, from within Australia, 2009

Result	Specimens from AFP cases involving children < 15 years of age	Specimens from AFP cases involving patients ≥15 years of age	Specimens from sources other than AFP	Total
Sabin poliovirus type 1	0	0	1	1
Non-polio enterovirus	2	0	61	63
Rhinovirus	0	0	5	5
No enterovirus identified	52	4	17	73
Total	54	4	84	142

AFP Acute flaccid paralysis.

amplified by RT-PCR. The virus was identified by nucleotide sequence alignment and phylogenetic analysis as Coxsackievirus B3. A second NPEV was identified from a faecal specimen of an AFP case from a patient with onset of paralysis in New South Wales in June 2009. The NPEV was identified as Coxsackievirus A4. Both cases had only 1 specimen referred for virus culture.

No enterovirus was isolated from the remaining 52 specimens.

Fifty of the total specimens received were from 29 cases with onset of AFP in 2009. Sufficient clinical information was available for all the AFP cases with specimens referred to be classified by the PEC as non-polio AFP. Four specimens were received in January 2009 from 2 AFP cases with onset of symptoms in December 2008; 1 specimen from 1 case and 3 specimens from the other.

Two specimens each were received from 2 cases involving patients aged 15 years or over, which was outside of the WHO AFP surveillance criterion. No enterovirus was isolated from the 4 specimens.

Sources other than acute flaccid paralysis

Echovirus 30 was identified from a non-AFP case aged over 14 years. An additional 4 specimens were referred from non-AFP cases, all of which were found to be enterovirus negative.

The Enterovirus Reference Laboratory Network (ERLNA) was established through seed funding from the Department of Health and Ageing for extended polio surveillance. A total of 89 samples (specimen extracts and nucleic acid extracts) were referred through the network (Table 4). The serotypes of 60 enteroviruses were identified by sequencing a fragment of the VP1 genomic region using 'CODEHOP' methodology.¹⁰

In October 2009, an uncharacterised poliovirus was referred through the ERLNA. A diagnostic virology laboratory in Victoria isolated the poliovirus from a faecal specimen of an unimmunised 1-month-old infant admitted for a respiratory infection with no indication of AFP. The virus was initially identified as an enterovirus by cytopathic effect in culture and subsequently as a poliovirus by immunofluorescence. The virus was identified as Sabin-like by the WHO diagnostic rRT-PCR ITD tests and the nucleotide sequence for the VP1 genomic region showed 905/906 (99.9%) nucleotide sequence identity to Sabin 1 prototype. A second specimen from the infant was requested to determine if virus shedding was prolonged but was not received. The source of the Sabin virus remains unknown.

Polio serology

Poliovirus serology is only performed for cases with a clinical suspicion of acute poliovirus infection. Nineteen requests for polio serology were received by the laboratory in the reporting period. All tests were cancelled after discussion with the referring doctor, as the requests were made to determine the patient's immune status for work or travel purposes.

Regional reference laboratory activities

In addition to the Australian samples, 206 specimens and virus isolates were received from various countries of the Western Pacific Region in 2009.

- Twenty-six faecal specimens from 14 AFP cases were referred from Pacific Island countries. Seven NPEVs were isolated from the specimens.

Table 4: Summary of enterovirus testing at the Australian National Poliovirus Reference Laboratory, referred from within Australia, 1995 to 2009

Year	Poliovirus		Non-polio enterovirus	No enterovirus detected	Total samples tested
	Sabin-like	Non-Sabin-like			
1995	190	0	200	13	403
1996	224	0	198	9	431
1997	124	0	76	0	200
1998	52	0	15	4	71
1999*	60	1	9	9	79
2000	45	0	44	47	136
2001*	46	5	33	75	159
2002	36	0	21	49	106
2003	9	0	15	47	71
2004	6	0	26	61	93
2005	18	0	10	39	67
2006	2	0	6	71	79
2007†	0	2	32	115	149
2008	0	0	20	92	112
2009‡	1	0	63	78	142

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

† Wild poliovirus type 1 was imported from Pakistan.

‡ Includes samples received for testing via the Enterovirus Reference Laboratory Network of Australia.

- Fifty-four faecal specimens from 33 cases of AFP were referred from Papua New Guinea. Eleven NPEVs were isolated from the specimens.
- Thirty-eight specimens and isolates were received from Malaysia including 18 polioviruses referred for intratypic differentiation.
- Eighty-eight specimens and isolates were received from the Philippines including 16 polioviruses referred for intratypic differentiation.

During 2009, The NPRL was invited to participate in the field evaluation of the poliovirus ITD rRT-PCR test kits by the CDC during the development and evaluation phases over an 18-month period. Subsequently the WHO requested the NPRL to host a regional training workshop on rRT-PCR techniques, in August 2009. The NPRL hosted facilitators from the CDC, WHO Headquarters, WHO Western Pacific Regional Office and an observer from the DoHA. The workshop participants were from Australia, China, Japan, Malaysia and Singapore.

Quality assurance program

The NPRL completed the WHO poliovirus ITD rRT-PCR proficiency panel in November 2009 and was the first polio reference laboratory to be fully accredited in the technique in the Western Pacific Region.

The NPRL also successfully completed the WHO poliovirus isolation and identification proficiency testing panel, which uses a revised testing algorithm introduced in endemic regions in 2006. The new algorithm is designed to shorten the time for issuing virus isolation reports from 28 days to 14 days and poliovirus ITD reports from 14 days to 7 days.

Discussion

Australia reported a non-polio AFP rate of 1.7 cases per 100,000 children less than 15 years of age in 2009. This exceeds the WHO AFP surveillance performance indicator of 1 non-polio AFP case per 100,000 children less than 15 years of age, which is an international standard to assess the sensitivity of a national AFP surveillance program. Australia has reached the WHO AFP surveillance performance indicator in 5 other

years since the program was established in 1995: 2000, 2001, 2004, 2006 and 2008. It should be noted, however, that while the national AFP surveillance scheme targets an age group at high risk of poliovirus infection, persons of any age are a potential source of a wild poliovirus importation, as evidenced by the 2007 importation involving a 22-year-old student from Pakistan.⁹

Four states (New South Wales, Tasmania, Victoria and Western Australia) achieved the AFP surveillance performance indicator rate in 2009. Victoria reported 2.2 non-polio AFP cases per 100,000 children less than 15 years of age, one of the highest rates ever reported for any jurisdiction in Australia and a vast turnaround from the consistent under-reporting for many years by that state. The introduction of the Paediatric Active Enhanced Disease Surveillance (PAEDS) scheme at a sentinel hospital in Victoria was a decisive factor in this result, with 21 of the 22 cases notified via the PAEDS system.¹¹

Queensland and the 2 territories, the Australian Capital Territory and the Northern Territory, did not reach the non-polio AFP indicator rate in 2009. Queensland is the only jurisdiction in Australia where AFP in children is a notifiable condition.

Despite the introduction of the PAEDS scheme in 2007, with hospital-based nurses to ascertain AFP cases and arrange for the referral of specimens to the NPRL, Australia has still never reached the WHO AFP surveillance performance indicator for the collection of adequate faecal specimens from 80% of AFP cases. One of the difficulties in achieving this target is the strict definition of adequate specimens: 2 faecal specimens collected more than 24 hours apart and within 14 days of the onset of paralysis. In 2009, 16 cases (33%) had adequate faecal collections, while a further 11 cases (23%) had only 1 adequate specimen collected, making a total of 56% of cases with at least 1 specimen collected within 14 days of the onset of symptoms.

No poliovirus was isolated from the specimens referred to the NPRL from AFP cases. A NPEV was isolated from each of the faecal specimens referred from 2 AFP cases: a coxsackievirus B3 was isolated from 1 case and a coxsackievirus A4 from the other. The establishment of the ERLNA by the NPRL provides another means of surveillance for poliovirus: virological surveillance rather than clinical surveillance for cases of AFP. The referral of an uncharacterised poliovirus from an unimmunised infant admitted to hospital with a respiratory infection through the ERLNA in

October 2009, was a significant result underscoring the usefulness of the reference network. Laboratories interested in collaborating with the ERLNA are encouraged to contact the NPRL for details.

Australia, along with the other countries of the Western Pacific Region, was declared free of indigenous wild poliovirus in 2000 and ceased usage of the Sabin 'live', attenuated oral polio vaccine in November 2005. The virus from the infant was typed as a Sabin type 1 poliovirus with a single mutation in the VP1 genomic region. The 99.9% sequence identity of the isolated virus to Sabin 1 prototype sequence indicates the virus was likely to have originated from a recent immunisation event, since poliovirus accumulates ~1% nucleotide mutations per year of replication.¹² The source of the Sabin poliovirus was never established but is likely to have been an importation from a country using OPV. The result demonstrates the ongoing risk faced by Australia of poliovirus importation, both wild and vaccine strains, since more than 90% of poliovirus infections are asymptomatic.

The introduction of the WHO poliovirus diagnostic real time RT-PCR at the NPRL will enable faster characterisation of untyped polioviruses compared with the previous end-point RT-PCR methodology. The real time RT-PCR assay includes an additional protocol to screen for vaccine derived polioviruses; Sabin strains with greater than 1% mutations compared with prototype sequence that is indicative of long-term viral replication and potentially person-to-person transmission. The NPRL will continue to sequence the full VP1 genomic region of all polioviruses referred to the laboratory, no matter what their source, as an additional precaution to further characterise all polioviruses. Laboratories identifying poliovirus from any patient of any age within Australia are requested to immediately contact the NPRL to arrange further identification of the virus.

Globally in 2009, a total of 1,604 cases of AFP were reported with 1,256 cases occurring within endemic countries. A total of 348 cases of AFP were reported in 23 non-endemic countries with Chad, Sudan and Guinea reporting 64, 45 and 42 cases respectively. These cases were related to the importation of wild poliovirus from Nigeria, which reported 388 cases in 2009.¹³ Significant progress has been made in Nigeria in 2010 with only 6 cases of AFP reported as of 18 June compared with 346 cases reported during the same period in 2009. The Tajikistan outbreak in 2010, with 413 reported cases of AFP and 19 deaths

due to wild poliovirus,³ is a salient reminder of the need for continued surveillance and vaccine coverage.

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