

# LABORATORY ACUTE FLACCID PARALYSIS SURVEILLANCE IN MALAYSIA: A DECADE OF COMMITMENT TO THE WHO GLOBAL POLIO ERADICATION INITIATIVE

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**Abstract.** The Institute for Medical Research, Malaysia, was designated the National Reference Laboratory for Poliomyelitis Eradication (NRLPE) in 1992. Since then, our Polio Laboratory has collaborated actively with the Disease Control Division, Ministry of Health (MOH), Malaysia and WHO towards achieving polio eradication. Since 1992, the NRLPE has investigated 1,063 stool specimens from 641 acute flaccid paralysis (AFP) cases. One hundred and one enteroviruses were isolated from these specimens. Positive cell cultures were confirmed by microneutralization assay using standard WHO antisera. All enterovirus isolates were sent to the Victorian Infectious Disease Reference Laboratory in Melbourne, Australia, for further identification and poliovirus intratypic differentiation. Thirty-one out of these 101 virus isolates (30%) were polioviruses (PV) and the remaining 70 (70%) were non-polio enteroviruses (NPEV) which included coxsackie B viruses, echoviruses and enterovirus 71. Three of the poliovirus isolates were wild-type polioviruses isolated in 1992 which were the last wild-type polioviruses isolated in Malaysia. The rest were vaccine-related Sabin-like strains. Monthly reports of the virological investigation of AFP cases are sent to WHO and to the MOH, AFP control committee. The NRLPE continues to play an integral role in AFP surveillance and is committed to the WHO's goal of global polio eradication by the year 2005.

## INTRODUCTION

Acute poliomyelitis is a viral infection of the anterior horn cells within the spinal cord, causing varying degrees of flaccid muscle paralysis. It can strike at any age, but over 50% of cases are mainly children under three. In most severe cases, polio can lead to death by asphyxiation. Eradication of polio is feasible because polioviruses only infect humans, with no animal reservoir. The virus cannot survive long in the environment without a host and effective vaccines are available.

In May 1988, the World Health Assembly committed WHO to eradicate poliomyelitis by the year 2000, a goal which has been extended to the year 2005. WHO's commitment has achieved remarkable success worldwide. Polio cases have decreased by 99.8% since 1988, from an estimated

350,000 cases to 600 in 2001. Polio eradication strategies rest on two main activities: immunization coverage and surveillance of acute flaccid paralysis (AFP) cases (CDC, 2001).

Immunization has been the key factor in forcing polio into retreat. Surveillance is equally important as immunization for the polio eradication initiative. The WHO has developed a surveillance system based on the detection, reporting and clinical and virological examination of all AFP cases under 15 years of age. A country's surveillance system should be sensitive enough to detect at least 1 case of AFP for every 100,000 children under 15, even in the absence of polio. In its early stages, polio may be difficult to differentiate from other forms of AFP, such as Guillain-Barré syndrome, transverse myelitis or traumatic neuritis. Laboratory isolation of wild poliovirus in stool specimens is important to differentiate vaccine-associated paralytic poliomyelitis and other forms of paralytic syndromes caused by other enteroviruses, emphasizing the need for an efficient and reliable laboratory sys-

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tem (Adas, 2001).

In Malaysia, oral polio vaccine was licensed and introduced into the childhood immunization program in 1972. Since then, the incidence of poliomyelitis declined with no reported cases between 1986 and 1991 and from 1993 to date. The Virology Unit at the Institute for Medical Research, Malaysia has been involved in Poliovirus and Enterovirus surveillance and research since the early 1970s. In 1992, the laboratory was officially designated by the WHO as the National Reference Laboratory for Poliomyelitis Eradication (NRLPE) and part of the Global Polio Network. The polio laboratory carries out virological investigation of all AFP cases and collaborates actively with the Disease Control Division, Ministry of Health and WHO to achieve polio eradication.

## MATERIALS AND METHODS

### Samples

Between 1992 and July 2002, the NRLPE investigated 1,063 stool specimens from 641 AFP cases sent from government hospitals throughout Malaysia. The specimens were accompanied by an AFP notification form with details of patient personal and clinical history. All stool specimens were processed (Fig 1) with chloroform before inoculation into RD-A and L20B cell lines from our laboratory stock held in liquid nitrogen at low passage. The cell cultures were grown in monolayers in 72 cm<sup>2</sup> tissue culture flasks in Earles Basal medium, Eagles (BME) supplemented with L-glutamine, Hepes buffer, pH 7.2, sodium bicarbonate, 10% fetal calf serum, 100 IU/ml penicillin G and 100 µg/ml streptomycin sulphate. Cells from 4- or 5-day confluent cul-

tures were resuspended in maintenance medium and concentration adjusted to  $6 \times 10^5$  cells/ml for use in the assay. Inoculated cell cultures were examined daily for cytopathological effect (CPE). The protocols for pre-treatment of stool specimens and virus isolation by cell culture followed WHO (2001) standard procedures (Fig 1).

### Microneutralization test procedure

Positive cell cultures were confirmed by microneutralization assay using standard WHO antisera. 0.05 ml samples of WHO standard enterovirus antiserum pools were distributed into wells of a microtiter plate followed by 0.05 ml of virus dilutions from  $10^{-1}$  to  $10^{-7}$ . The plates were covered and incubated at 36°C in a CO<sub>2</sub> incubator for 1 hour. After incubation, 0.1 ml of cell culture suspension (approximately  $1.5 \times 10^5$ ) were added to all wells. The antiserum pools that prevent the development of CPE indicate the identity of the virus isolate. Enterovirus isolates were sent to the Victorian Infectious Disease Reference Laboratory in Melbourne, Australia, for further identification and poliovirus intratypic differentiation.

### Controls

The controls incorporated in the assay included cell control to check for normal cell morphology and virus control to detect virus infectivity with complete CPE. Back titration was included to confirm the amount of virus used in the assay was within the acceptable range.

## RESULTS

One hundred and seven enteroviruses were isolated from the 1,063 stool specimens investigated between 1992 and July 2002 (Table 1).

Table 1  
AFP isolates between 1992 and July 2002.

Year	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002
No. AFP cases	10	15	16	17	32	77	86	85	147	108	63
No. stool specimens	14	28	28	30	44	118	147	130	250	146	128
Enterovirus isolate	4 PV	1 PV 6 NPEV	1 NPEV	4PV 6 NPEV	3PV 2 NPEV	6PV 6 NPEV	4PV 4 NPEV	4 NPEV	6PV 18 NPEV	3PV 19 NPEV	5PV 4 NPEV

Thirty-six out of 107 virus isolates were polioviruses (PV) and the remaining were non-polio enteroviruses (NPEV) which included coxsackie B viruses, echoviruses and enterovirus 71 (Fig 2). Out of 36 polioviruses isolated, 3 were wild type isolated in 1992, caused by importation of wild polioviruses that originated from the Indian subcontinent. The wild-type polioviruses were confirmed by intratypic differentiation by the Centers for Disease Control, Atlanta, USA. The remaining were vaccine-related Sabin-like strains. Out of these, 11 were Sabin type 3 viruses, 10 were Sabin type 2, 10 were a mixture of Sabin 2 and 3, and 4 were Sabin type 1.

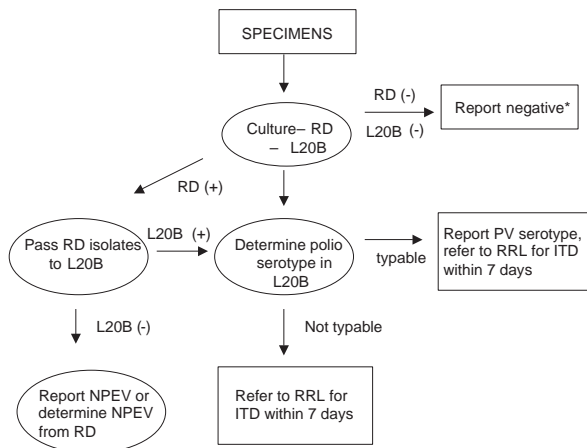


Fig 1—Flow chart for laboratory investigation of AFP.

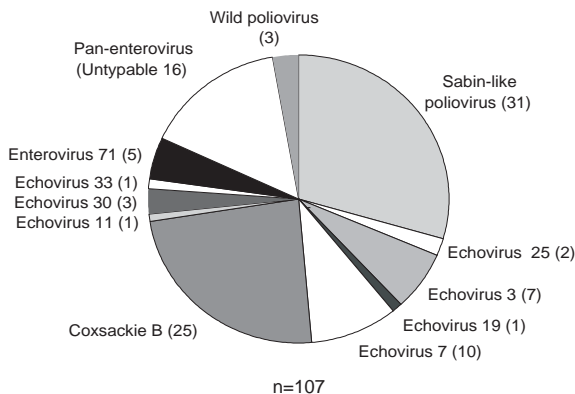


Fig 2—Enterovirus isolates from acute flaccid paralysis cases.

## DISCUSSION

The primary goal of AFP surveillance for poliovirus eradication is to promptly detect possible areas of circulating wild poliovirus to implement control measures. No wild poliovirus has been identified in Malaysia since 1993. However, it remains essential that surveillance is maintained until global eradication is achieved because of the risk of wild virus being imported from endemic regions.

Thirty-four out of 107 enteroviruses isolated between 1993 and 2002 were poliovirus, vaccine-related Sabin strains. In AFP cases, isolation of these strains indicates vaccine-associated paralytic polio (VAPP), a rare adverse event following ingestion of live oral poliovirus vaccine. The mechanism of VAPP is believed to be a mutation or reversion of the vaccine virus to a more neurotropic form. Reversion is believed to occur in almost all vaccine recipients, but only rarely results in paralytic disease and is more likely to occur in immunodeficient children. Studies have reported that Sabin type 3 and 2 viruses have a greater tendency to revert to a neurovirulence on passage in the human gut (Evans *et al*, 1985). Seventy-one of the enteroviruses were non-polio enteroviruses, which are known to circulate in all populations and are associated with a vast range of presentations, from asymptomatic to acute flaccid paralysis resembling polio (Dietz *et al*, 1995). Our data showed that case reporting by AFP surveillance and subsequent laboratory diagnosis are essential to evaluate the interruption of wild polio. A high level of laboratory surveillance has shown that no outbreaks of wild polio have occurred since 1992, rather, vaccine-associated paralytic polio and infections with non-polio enterovirus were detected during this period.

Monthly reports of the virological investigation of AFP cases are sent to the WHO and to the Ministry of Health, AFP review committee. Proficiency testing to assess the quality of work in the laboratory commenced in 1993, and continues annually. This is monitored externally by the WHO Regional Reference Laboratory in Melbourne, Australia. The National Polio Laboratory has achieved the Quality Indicators set by WHO to reach international standards required

for certification of poliomyelitis eradication, as evidenced by the accreditation status awarded by the WHO in the Global Polio Laboratory Network for the years 1998 to 2002. The NRLPE continues to play an integral role in AFP surveillance and is committed towards WHO's goal of polio eradication by the year 2005.

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