



POLIO LAB NETWORK

Quarterly Update



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Poliovirus type 2 (MEF-1) found in northern India

In October 2000, the Lucknow National Polio Laboratory (NPL) reported the isolation of wild poliovirus type 2 from stool samples collected in September 2000 from 3 AFP cases in eastern Uttar Pradesh (2) and Bihar (1). Sequencing by the Enterovirus Research Center (ERC), Mumbai found no relation of these isolates to the wild type 2 viruses that last circulated in India in 1999, but a virtual identity with the common laboratory type 2 reference strain MEF-1 (see accompanying article). A thorough investigation revealed no source of MEF-1 in the highly competent Lucknow laboratory, but in the absence of any other plausible explanation, the isolations were attributed to laboratory contamination of unexplained origin.

Two years later, in December 2002, ERC reported the isolation of MEF-1 from 3 AFP cases from western Uttar Pradesh. Laboratory contamination was again suspected, although considered most unusual. Nevertheless, MEF-1 had been a frequent contaminant world wide in the past, often in laboratories unaware of its presence. When ERC reported MEF-1 isolates from 3 more AFP cases in Uttar Pradesh in January, high on the list of suspected sources for contamination was the Sabin-like antiserum distributed by RIVM, Bilthoven for the ELISA intratypic differentiation test (ITD). The type 2 reagent was prepared by cross adsorption with MEF-1 and some lots had been found to contain traces of virus (*Quarterly Update*, v. IX, #1, 2003, p3).

This lead proved to be incorrect based on 4 observations. First, the national and international reviewers who had been invited to visit ERC found no evidence for laboratory contamination. Second, further testing confirmed the presence of MEF-1 in the original stool samples. Third, also in March, the Ahmedabad NPL independently reported the recovery

of type 2 from still another case, this time in the State of Gujarat, and identified by ERC as MEF-1. Fourth, the original Gujarat stool sample, which had never been in ERC, was confirmed by RIVM as positive for MEF-1.

All evidence indicated MEF-1 was present in the stool samples at the time of arrival in the laboratories. Contamination en route to the laboratories was unlikely, as stool samples were collected by different

Editorial Note: On March 21, 2003, the Weekly Epidemiological Record (WER) (12, 2003, 78, 88) reported the isolation of the wild type 2 reference strain MEF-1 from AFP cases in northern India. Almost five months later, on August 8, the WER (32, 2003, 78, 284) reported that MEF-1 had been recovered from one batch of OPV distributed in the same area. Behind these two brief reports was an intensive investigation by the Government of India (GOI) and WHO. As is characteristic of the polio eradication initiative, the investigation was an outstanding collaborative effort between national and international field, epidemiology, and laboratory staff. In this issue of UPDATE we provide a brief (with apologies to the field and epidemiology staff) laboratory report to keep Network collaborators informed of the increasingly complex application of current technology. A complete description of all aspects of the investigation will be published elsewhere in time.

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people in different locations, and reached the laboratories by three different routes. Clearly, MEF-1 had been reintroduced into the population, and at a time of intense supplemental immunization activities.

Genomic sequencing was performed by ERC and RIVM on 11 MEF-1 isolates. Two were from the AFP cases in 2000 (the authenticity of the stool from the 3rd AFP case was questionable). Four were from the AFP cases in 2002. Five were from 2003 and consisted of 3 isolates from the AFP cases and one each from a healthy child and an environmental sample. Sequence data from the VP1 gene revealed all isolates belonged to one of 2 closely related strains of MEF-1 and all were within 2 nucleotide substitutions of the consensus sequences for the 2 strains. More importantly, 4 of the isolates from 2000 (1) and 2003 (3, including one from an environmental sample) were identical. These four isolates and one from 2002 had G at VP1 position 223, constituting a unique marker for identifying one of the specific MEF-1 strains involved. There was no mutational evidence of circulation in the community, with the possible exception of the Gujarat isolate and one December 2002 isolate from Uttar Pradesh. Taken together, these laboratory findings strongly suggested multiple introductions into the population from a single source, with only limited spread.

An extensive investigation within the affected region by the Government of India and WHO in March through June 2003 revealed no potential sources of MEF-1 in diagnostic and research laboratories, or vaccine bottling facilities, nor opportunities for intermittent contamination from laboratory waste materials. Simultaneously, batches of trivalent OPV thought to have been used in the affected communities were identified and collected from the field and control authorities and shipped to the National Institute for Biological Standards (NIBSC), UK, RIVM, and ERC. Using biologic assays with Sabin-specific type 2 antiserum in the presence of type 1 and 3 antisera (or specific monoclonal antibodies), or direct PCR tests using different MEF-1 specific primers, all three laboratories in late July independently found MEF-1 in the same batch of OPV. The breakthrough virus in biological assays had the characteristics of wild poliovirus, e.g., non-Sabin-like in ELISA and growth at 40.5 degrees. Sequencing confirmed the breakthrough virus to be

identical to the stool isolates, with the majority virus having the unique 223G marker. Tests of all other batches used in the area have been negative to date.

The investigation into how a single OPV batch was contaminated with MEF-1 in 2002, and possibly in 2000, is expected to continue for some time, but several general observations can be made. First, it is unclear during this time of intense immunization in Western Uttar Pradesh just how many of the 9 AFP cases could be attributed to MEF-1. On several occasions the vaccine was given after onset of paralysis and days before stool samples were collected. Second, and very importantly, no further isolations have been made of MEF-1 from AFP cases in India since February 2003. Third, this investigation was successful because of the high quality field and laboratory surveillance and vaccination programs in India. There was little evidence of AFP cases being missed, case investigations were thorough and stool samples were transported and tested promptly. Cooperation at all levels was outstanding.

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Global databank of historical IPV seed and reference strains given high priority

The April 2003 Global Lab Meeting recommended that:

Historical strains of polioviruses that are used as reference strains or in vaccine production should be maintained in designated global specialized laboratories. Full sequence data on such historically important poliovirus strains should be available for program use.

The primary purpose of an inventory of full sequence data of all common poliovirus reference/IPV

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vaccine strains is to assure their recognition if isolated and facilitate investigations of any future incidents where contamination with these strains might be suspected. The crucial need for such information is underscored by the lab investigation into the origin of the poliovirus type 2 MEF-1 isolates in northern India (see accompanying article this UPDATE).

MEF-1 was first isolated from pooled central nervous system material from at least 2 poliomyelitis cases occurring in Alexandria, Egypt in 1942. Original isolations were made through spinal cord inoculation of a monkey, with subsequent adaptation to cell culture. The virus was widely distributed over the intervening years, and was the wild type 2 reference strain of choice for many applications in research and quality control laboratories. MEF-1 remains the type 2 component of inactivated poliovirus vaccine (IPV).

Current investigations have shown that some MEF-1 seed stocks consist of two very closely related strains, consistent with the history of MEF-1 isolation from pooled materials. Other small differences in the genomic sequence of historic virus stocks might be expected because of the normal accumulation of mutations associated with different passage histories in the laboratory. Activities are currently underway at the National Institute for Biological Standards, Potters Bar, UK, to examine registered MEF-1 seed stocks from all current IPV producers to determine the extent of genetic variation present.



PEI loses two giants

Fredrick C. Robbins, well known and much admired in the field of polio, died in early August. He shared the Nobel Prize for demonstrating in the late 1940s that poliovirus could be grown in large quantities on cell culture, opening up extraordinary opportunities for research and leading to the development of polio vaccines. Fred chaired the Commission that certified the Americas to be polio-free in 1994 and served as a member of the Global Certification Commission.

Rafi Grigorievich Aslanian, a highly respected leader in the polio eradication effort, died on 5 Au-

gust. A career WHO staff member, he was an early enthusiastic supporter of polio eradication. He was co-founder of the innovative Operation MECACAR (Mediterranean, Caucasus, and Central Asian Republics) that coordinated EUR and EMR efforts in the countries with the highest polio rates in the two Regions. Rafi retired from WHO, EMRO in 1999, but remained dedicated to eradication, serving as a consultant in several Regions and playing a key role in the certification of EUR as polio-free in 2002.



Wild viruses not needed for neutralization tests

In the implementation of the survey and inventory phase of wild poliovirus containment, laboratories have been asked to destroy wild polioviruses or replace them with Sabin strain viruses if needed. A few laboratories have justified retaining wild polioviruses on the grounds that Sabin viruses were considered to be less sensitive for neutralization tests (NT) serosurveys. However, NT serosurveys have not played an important role in the eradication effort. Serosurveys are often regarded as resource intensive, poorly standardized, and not timely enough for a fast moving initiative that relies on vaccine coverage and the absence of polio to evaluate progress. In fact, NT serosurveys are so seldom used within the Network that the NT is not even included in the current WHO Laboratory Manual.

There have been special situations, research projects, and studies on vaccine immunogenicity where the NT proved useful. In almost all circumstances the use of Sabin strains is recommended. A panel convened by WHO in 1991 recommended the use of Sabin strains for NT primarily on the grounds of improving test comparability through standard reagents. In addition to avoiding wild virus strains, Sabin strains were also recognized as the antigens of choice for evaluating immune responses to Sabin oral polio vaccines. Any theoretical advantages of using wild polioviruses rather than Sabin strains must be weighed against the known programmatic and containment risks of working with wild polioviruses in the laboratory.



Poliovirus Surveillance Report, January - June 2003

REGION	Number of AFP cases with specimens	P1 only	P2 only	P3 only	Polio mix	Polio + NPEV	NPEV only	Pending culture results	% Positive for NPEV	% Results within 28 days			
AFR	3,649	57	15	83	17	19	492	16	14%	98%			
AMR	762	3	5	7	2	119	102	105	16%	74%			
EMR	2,615	61	21	60	37	6	455	38	15%	95%			
EUR	1,039	11	18	16	15	0	103	268	5%	89%			
SEAR	4,668	131	43	103	107	33	896	2	15%	99%			
WPR	2,229	17	31	28	27	7	151	390	7%	92%			
Regional Ref. Lab	Poliovirus Intratypic Differentiation Results												
	No. of cases with isolates submitted	Type 1			Type 2			Type 3			Pending		% AFP cases with ITD results within 14 days
Wild		Sabin	VDPV	Wild	Sabin	VDPV	Wild	Sabin	VDPV	ITD Discordant results	ITD Tests Pending*		
AFR	213	56	30	0	0	33	0	64	50	0	0	0	98%
AMR	15	0	3	0	0	5	0	0	7	0	0	0	—
EMR	185	32	57	0	0	45	0	17	73	0	0	13	82%
EUR	172	0	10	0	0	10	1	0	20	0	0	3	—
SEAR	417	80	148	0	0	96	0	10	195	0	0	9	85%
WPR	125	0	37	0	0	56	0	0	63	0	18	3	77%

* No. cases with any isolates pending completion of ITD tests

† Only for isolates designated as VDPV from sequencing of VP1 gene

AFR: Between January and June 2003, wild viruses were detected from AFP cases in the countries of Nigeria (90), Niger (2), and Ghana (3). This compares with the detection of wild viruses in 4 countries in 2002; Nigeria (202), Niger (3), Zambia (2) and Burkina Faso (1). Sequence and epidemiological data suggested that viruses detected from Zambia and Burkina Faso were imported from Angola and from a Nigeria/Niger reservoir, respectively. Additionally in 2002, VDPV type 2 were detected in 4 AFP cases in Madagascar (all had onset between March and April), 1 case in Nigeria (with onset in September).

AMR: No wild polioviruses were detected between January and June 2003. No VDPV have been detected since the type 1 cases were reported from Haiti and Dominican Republic in the first half of 2001.

EMR: Wild polioviruses were detected in AFP cases from 5 countries between January and June 2003: Afghanistan (1), Egypt (1), and Pakistan (41). Testing of sewage samples as part of a supplementary surveillance activity revealed 5 wild type 1 polioviruses in sewage samples collected from 4 settlements in Egypt. In 2002 wild polioviruses were isolated from AFP cases in Afghanistan (10), Egypt (7), Pakistan (90), Somalia (3) and from 26 sewage samples from 10 settlements in Egypt.

EUR: This region was declared free from endemic wild poliovirus circulation in June 2002. In 2002 a type 3 VDPV was isolated from a sewage sample from Estonia. Between January and June 2003, type 3 VDPVs were isolated from an AFP case in Kazakhstan and a single sewage sample from Slovakia.

SEAR: India remained the only country with wild polioviruses detected in the region. Ninety wild virus confirmed AFP cases were detected between January and June 2003, compared to 1600 cases detected between January and December 2002.

WPR: This region has been free from endemic wild poliovirus circulation since March 1997. In 2002, VDPVs had been isolated from 2 AFP cases in China (1 type 1 and 1 type 3) through retrospective testing of isolates. Additionally, between January and June 2003, a type 1 VDPV was isolated from a single, healthy, non paralysed, child in Mongolia.

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