

VIROLOGIC SURVEILLANCE OF POLIOMYELITIS IN DELHI

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Objective: Virologic surveillance of poliomyelitis to monitor the transmission of wild polio virus in the community. **Study area:** All major hospitals of Delhi and surrounding area. **Methods;** Stool samples were collected from 1221 cases of acute flaccid paralysis during 1992-1994 and were subjected to virus isolation on RD and HEp₂ cell line. Viruses isolated were analyzed further by microneutralization test using polio and nonpolio antisera. The polio isolates were further characterized as vaccine or wild type using ELISA and probe technology. **Results:** Out of the 1221 cases tested, virus was isolated in 57.4%. Among the virus positive cases, polio was isolated in 57% and in 43% non polio enteroviruses were detected. The most prevalent was polio virus type 1. Most of the strains were wild type. **Conclusion:** Wild polio virus was prevailing in the community under study between the years 1992-1994.

Key words: Acute flaccid paralysis, Intratypic differentiation, Wild polio virus.

PARALYTIC poliomyelitis is on the threshold of extinction following the World Health Assembly alliance for polio eradication by 2000 AD(1). The eradication of endemic poliomyelitis requires the extinction of wild polioviruses(2). Virologic surveillance, which involves isolation of virus followed by serotyping and intratypic differentiation of virus as wild or vaccine is an important activity in this context. Virologic surveillance is the primary means for understanding and detecting poliovirus transmission in the country and differentiating from other causes of acute flaccid paralysis (AFP) like Gullian Barre syndrome and infection due to non-polio enteroviruses(3). As the immunization coverage increases, cases become more sporadic and the role of virologic studies assumes greater importance. Three types of polio viruses have been known. Attenuated strains or Sabin strain were derived from wild type isolates. These attenuated strains possess

greatly reduced neurovirulence and are different from the wild strains(4). Based on these differences, there are various intratypic tests developed to differentiate wild from vaccine strains(5). We present here, the detailed analysis of virus isolation from the year 1992-1994 in cases of AFP from Delhi.

Subjects and Methods

Stool samples collected by rectal tubing technique were received from all major hospitals of Delhi between 1992-1994. These cases were suffering from AFP (includes many cases of typical presentation of acute paralytic poliomyelitis). From each subjects two samples were planned to be collected at an interval of 24-48 hours and preferably within 15 days of onset of paralysis but in almost 70% of cases only one sample was collected. Each sample after pre-treatment was subjected to virus isolation on to RD and HEp₂ cell line as per the

WHO guidelines(6). The isolates were typed as polio type 1,2,3 or mixture using antisera procured from RIVM, Netherland and standard techniques were followed(6). All non-polio virus isolates were further subjected to enterovirus typing using pool antisera procured from RIVM, Netherland and by standard procedure(6).

Polio virus strains representing each year from 1992-1994 and each polio type 1, 2 and 3 were randomly selected for intratypic differentiation representing every month isolates. Almost 70% of isolates were subjected to this test. Due to shortage of reagents, all the strains were not subjected to intratypic differentiation. As per WHO Standard guidelines, intratypic differentiation was done by ELISA technique using cross absorbed rabbit antisera(7) and also by RNA probe hybridization technique(8). For ELISA technique, the reagents were procured from RIVM Netherland and for RNA probe hybridization, reagents were procured from Molecular Virology Laboratory, CDC, Atlanta, USA. For hybridization procedures, two categories of probes *i.e.*, Group probe and polio virus genotype specific Sabin probe were used. In this technique, Sabin related strains were directly identified using Sabin strain specific probes. Wild polioviruses were

recognized by the inability of their genomes to form stable hybrid with the Sabin strain specific probes. As per the WHO criteria, the strain will be interpreted Sabin or wild after being tested by two techniques, one by ELISA and another by RNA Probe technology(7,8).

Results

During the years 1992-1994, a total of 1221 cases of AFP were subjected to virus isolation (*Table I*). Virus could be isolated only in 701 (57.4%) cases of which polio viruses were found in 399 cases (57.0%) while non-Polio enteroviruses or a mixture of polio and non-polio enteroviruses were seen in 43.0% of cases. The most prevalent polio virus was polio type 1 in 1992 and 1994 while in 1993, it was polio virus type 2. Those which could not be typed as polio by polio typing antisera were further typed as Coxsackie B or Echo or Coxsackie A, *etc.* viruses using enterovirus pool antisera (*Table II*). However, during 1993 and 1994, a mixture of polio and non-polio entero-viruses were found in 8 and 24 cases, respectively. These are the cases which could have been labeled as non-polio paralytic cases. All these case had actually polio myelitis but could have been missed if only polio typing was done.

TABLE I—Virus Isolations Among Laboratory Reported AFP Cases in and Around Delhi.

Year	Total cases	Polio positive	Polio virus type				Non-polio-enterovirus	Total-negative
			P1	P2	P3	Mix		
1992	577	202 (35.0)	163	12	11	16	185 (47.8*)	190 (33.0)
1993	267	76 (35.0)	15	34	6	21	41 (35.0*)	150 (56.1)
1994	387	121 (31.2)	88	9	18	6	76 (38.5*)	190 (50.9)
Total	1221	399	266	55	35	43	302	530

Figures in parentheses indicate percentages.

* Percentage calculated out of total cases showing positive virus isolations.

** Includes mixture of polio virus and nonpolio enterovirus.

Representative isolates in each year were subjected to intratypic differentiation by ELISA and RNA probe hybridization (Table III). Most of the representative strains of 1992, 1993 and 1994 of polio types 1, 2 and 3 were wild in nature. Only one strain of polio type 2 in 1993 and one each of type 2 and type 3 in 1994 were Sabin type. Variable results were obtained in one strain of type 1 and two strains of type 2 and type 3. These strains were referred to CDC Atlanta for further analysis.

Discussion

The World Health Assembly goal to eradicate poliomyelitis globally before the year 2000 AD still has many hurdles to overcome. Virologic surveillance is instrumental in monitoring the result of intervention(9). Virologic surveillance

includes identification of polioviruses by serotyping and identifying whether they are vaccine related or wild which has important implications from the epidemiologic and programmatic point of view. Thus early detection of community transmission of wild polio viruses will help us to take control measures in time(10). Keeping this in view, the present work on virologic surveillance was carried out. Virus was isolated only in 57.4% of cases of AFP during the period of study. The isolation rate was low due to the fact that only one stool sample was collected in 70% of cases and that too by rectal tubing technique. In few cases, the sample was collected only after 3 weeks or more of onset of paralysis. All these factors contributed to low isolation rate. This indicates the need to stress for proper collection of stool samples from every

TABLE II—Detailed Analysis of Non-Polio Enteroviruses Isolates

Year	Total type isolates	Coxsackie	Different echo viruses	Polio+Entero viruses	Mixture of two non polio entero viruses	Non-typable viruses
1992	25	7	7*	—	—	11
1993	25	7	3**	8	—	5
1994	76	5	29***	24	6	12

* E6(2) E2(2) E13(2) E33(1); ** E29 (1) E2(2)

*** E1(1) E3(2) E2\4(13) Es(1) E9(3) E11(1) E20(2) E27(2) E29(2) E33(2)

TABLE III—Intratypic Differentiation of Polio Virus Isolates (1992-1994) in Relation to Different Polio Virus Types

Intratypic Differentiation	Yearwise polio virus types								
	1992			1993			1994		
	1	2	3	1	2	3	1	2	3
Wild	10	10	10	4	12	9	25	9	16
Sabin	0	0	0	1	1	0	0	0	1
Others	0	0	0	0	0	0	1	2	2

case of acute flaccid paralysis. From every case of AFP, two stool samples should be collected in a plain vial received from the laboratory. The two samples should be collected at an interval of 24 to 48 hours within 15 days of onset of paralysis.

Since the seroconversion is most efficient for polio virus type 2(11), the detection of endemic polio type 2 virus indicates that coverage is not sufficient and there are pockets in the community with low immunization coverage. Detection of polio type 3 virus indicates that the level of population immunity is not sufficient to block the transmission. In the present study, during 1992 the most prevalent polio virus isolate was polio type 1 but in 1993 it was polio type 2 indicating that in Delhi and surrounding areas there are some pockets having low immunization coverage. However, in 1994, polio type 1 was the most prevalent type as was in 1992. This may indicate a fall in coverage in some areas. More steps need to be taken to increase the population immunity so as to replace even polio type 1 with polio type 3 and then eradication of polio virus.

Non-Polio entero viruses were isolated in a significant number of cases in the present study. About 30% of these isolates were actually a mixture of polio and nonpolio and these polio cases were missed in the polio typing. This fact stresses the need for more critical typing of viruses and laboratory investigations in cases of AFP(3). Moreover, isolation of virus from AFP patient is essential to establish if the case is due to wild or Sabin strain of polio virus. On Intratypic differentiation by ELISA and RNA probe hybridization, mainly wild type poliovirus was present indicating that wild polio virus transmission was prevalent in the community.

It needs to be stressed here that an effective polio surveillance consists of a

sensitive AFP surveillance system integrated with a high quality polio virus laboratory network. The basic criteria for certification of polio eradication is the absence of virologically confirmed indigenous poliomyelitis cases for a period of 3 years under circumstances of adequate surveillance and not the 60 days follow up of a case as was the usual belief(12). We have already witnessed the extinction of polio virus from many countries and more will be followed in the near future. For the final goal of global polio eradication a stronger link between epidemiologic surveillance/laboratory studies and programme manager is desirable.

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NOTES AND NEWS

CONFERENCE OF UP CHAPTER OF INDIAN ACADEMY OF PEDIATRICS

This event is to held on 26th-27th October 96 at L.L.R.M. Medical College, Meerut. The delegate fee for ordinary participants is Rs. 300/- (Rs. 350/- after 15.10.96), for postgraduates and accompanying persons a discount of Rs 50/- will be given. The last date for acceptance of abstracts of 200 words is 30.9.1996. For further details please contact: Dr. D.K. Sharma, Professor and Head, Department of Pediatrics, Chairman Organizing Committee or Dr. (Mrs.) S.P. Goel, Assistant Professor, Department of Pediatrics, Organizing Secretary, L.L.R.M. Medical College, Meerut 250 004, Tel.: (Resi.) - (0121) - 560777, 560666.

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This event is being organized under the auspices of IAP Jabalpur Branch on Sunday, 27th October 1996 at Jabalpur. For further details please contact Dr. Vrind Bhardwaj, Organizing Secretary, Pediatric Endocrinology Symposium, Department of Pediatrics, Government Medical College, Jabalpur (MP)482 003, Tel: 422117, Extn. 54 (Office); 29728 (Residence).