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Necessity of Two-Stool Sample Test for Sensitive Detection of Poliovirus

Tetsuo Yoneyama*, Hiromu Yoshida, Kumiko Yoshii, Junko Wada,
Hiroyuki Shimizu and Tatsuo Miyamura

*Department of Virology II, National Institute of Infectious Diseases,
Gakuen 4-7-1, Musashimurayama-shi, Tokyo 208-0011*

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By the initiative of a WHO polio-eradication program, the Western Pacific Region is now declared free of wild poliovirus circulation (1). However, wild polioviruses are still circulating in other restricted areas in the world (2). Consequently, prompt and accurate laboratory diagnosis as well as maintenance of high immunity levels are of critical importance until eradication of poliomyelitis is truly achieved at global level. As a basic strategy, the examination of two stool specimens from one acute flaccid paralysis (AFP) case within 2 weeks has been practiced in the global laboratory network for the program (3). If it were possible to reduce the number of stool collections from two specimens to one without losing diagnostic accuracy, the workload required for laboratory testing and surveillance would be reduced.

Since 1996, stool specimens from AFP patients in Lao People's Democratic Republic (Lao) and Cambodia (Cam) were directly sent to the National Institute of Infectious Diseases in Tokyo and were examined for the presence of polioviruses and non-polio enteroviruses (NPEVs). The last wild poliovirus (type 1) isolate was obtained from a specimen sent from Cambodia in 1997 (4).

We retrospectively analyzed the virus isolation data in our laboratory from 1998 to 2000 and investigated whether the two-stool specimen test actually increased the efficiency of

virus detection. NPEVs were isolated by using HEp-2 and RD cell lines, and polioviruses were detected by using the above two cell lines plus L20B cell lines.

Among 772 cases of AFP patients, NPEVs were isolated from 278 cases; 200 cases were detected in both of the two samples, 42 cases in only the first stool sample, and 36 cases in only the second stool sample. The isolation rate (36%) of NPEVs using three different cell lines, HEp-2, RD, and L20B cells far exceeded the WHO requirement (10%) (5). If the number of samples collected had been reduced from two to one, the detection rate of NPEVs would have been reduced from 278 cases to 242 or 236 cases, i.e., by 14%. As for poliovirus isolation, only 65% of the AFP cases (17 cases) were positive in both samples, and 31% of the AFP cases (8 cases) were poliovirus-positive in the second stool specimen only (Table 1). The latter poliovirus could have gone-undetected in the one-stool specimen protocol.

The above data suggest that a substantial number of poliovirus isolations might have been missed if the second stool specimens had not been examined. The polio outbreak associated with type 1 circulating vaccine derived poliovirus (cVDPV) in the Caribbean island of Hispaniola (6) was a quite unexpected, as that polio outbreak was first thought to be due to a re-introduction of wild polioviruses from other regions. That outbreak reaffirmed the importance of maintaining sensitive laboratory poliovirus surveillance for the possible occurrence of cVDPV as well as wild poliovirus outbreaks. The two-stool

*Corresponding author: Fax: +81-47-256-4729, E-mail: tyoneyam@nih.go.jp

Table 1. Virus Isolation from first (St1), second (St2), or both stools collected from AFP cases in Lao and Cambodia, 1998-2000

Country-Year	No. of AFP case	Results of NPEV isolation			Results of poliovirus isolation			No. of mix isolation (NPEV and poliovirus)	No. of negative cases in both stools
		St1+, St2+	St1+, St2-	St1-, St2+	St1+, St2+	St1+, St2-	St1-, St2+		
Lao-1998	77	18	5	4	1	0	1	0	48
Lao-1999	84	28	2	3	2	0	2	2	49
Lao-2000	71	18	6	4	3	0	1	1	40
Cam-1998	165	54	11	13	6	0	2	2	81
Cam-1999	178	34	13	3	3	0	1	0	124
Cam-2000	197	48	5	9	2	1	1	0	131
Total	772	200(72%)	42(15%)	36(13%)	17(65%)	1(4%)	8(31%)	5	473

specimen protocol, which significantly increases the sensitivity of poliovirus detection, should be maintained until the global eradication of poliomyelitis has been achieved.

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