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Contribution of second stool specimen to increased sensitivity of poliovirus detection in India, 1998–2000

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SUMMARY

Acute flaccid paralysis (AFP) surveillance data from India were analysed to examine sensitivity of poliovirus isolation from stool specimens and the added sensitivity obtained from collection of a second stool specimen. Analysis was restricted to Indian AFP cases, 1998–2000, with two adequate stool specimens. The proportion of cases confirmed with wild poliovirus isolation by the second specimen only was calculated, regardless of specimen quality. Overall specimen sensitivity (1998–2000) was 81% using the first specimen, 78% using the second, and 96% using both. Sensitivity increased from 1998 to 2000, with slightly higher sensitivity each year for the first specimen. The second specimen increased sensitivity by 15% overall and contributed more when the first specimen was collected late or was in poor condition. As wild poliovirus disappears, increased sensitivity provided by a second stool specimen may reduce the risk of missing circulating virus.

INTRODUCTION

The objective of the acute flaccid paralysis (AFP) surveillance system is to detect the presence of wild poliovirus by investigating all reported AFP cases. This allows for identification of areas with ongoing poliovirus transmission and implementation of appropriate vaccination responses. Since AFP surveillance began in India in 1997, the country has made rapid progress in controlling poliomyelitis, with the number of wild poliovirus cases decreasing from 1934 in 1998 to 265 in 2000 (National Polio Surveillance Unit, New Delhi). At the same time, the AFP surveillance system, which achieved certification level performance in 1998, continued to expand and improve. Non-polio AFP rate was $\geqslant 1$ per 100 000 under age 15 years and $\geqslant 80\%$ AFP cases with two adequate stool specimens. Adequate stool specimens were defined as two specimens collected $\geqslant 24$ h apart and within 14 days of paralysis onset, both arriving at a World Health Organization [WHO]-accredited laboratory in good condition (cold chain maintained, container not leaking, good specimen quality, and adequate quantity).

A critical component of the AFP surveillance system is the collection, from each AFP case, of two stool specimens within 14 days of paralysis onset for poliovirus isolation and intratypic differentiation. Early investigations revealed that additional specimens could increase poliovirus identification rate by 10%

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[1], and it was subsequently recommended that two stool specimens be collected from every reported AFP case. The contribution of the second stool specimen to the sensitivity of poliovirus isolation must be weighed against the considerable resources required to collect and process a second specimen from every AFP case. In the end stage of polio eradication in the region of the Americas, for example, it was determined that routine processing of only one stool specimen from each AFP case would be sufficient to detect areas of poliovirus transmission while not overburdening laboratories [2].

As eradication nears, it is crucial to improve our understanding of how AFP surveillance can provide reliable data to support the apparent absence of wild poliovirus transmission, and the importance of sensitivity increases substantially. As the eradication programme progresses, more situations will arise in which a second stool specimen could provide decisive evidence for the presence or absence of poliovirus transmission in an area. In this report, we use surveillance data from India (1998–2000) to examine the sensitivity of poliovirus isolation from stool specimens and the added sensitivity obtained from collection of a second stool specimen. We describe changes in stool specimen sensitivity for poliovirus isolation during a time when wild poliovirus transmission has been rapidly declining.

METHODS

AFP surveillance system, India

Active surveillance for acute flaccid paralysis (AFP) was established in India in October 1997. International performance standards were met and then surpassed starting in May 1998. AFP was defined as any case of acute-onset flaccid paralysis in a child less than 15 years of age without another obvious cause, or any case of paralytic illness occurring in any person in whom poliomyelitis is suspected, regardless of age [3]. In India, AFP cases were detected by active surveillance with over 8500 healthcare institutions reporting weekly in 2000 [4].

Epidemiological and clinical information was collected both at an initial investigation and 60 days after paralysis onset for every AFP case identified and reported to the surveillance system. The protocol required all AFP cases to be investigated within 48 h of being reported. Investigators confirmed that the case was AFP and completed a standard case investigation form. Data collected as part of the investigation included date of paralysis onset, age of the child, immunization history, clinical history and findings (signs and symptoms). Investigators also arranged for stool specimen collection and shipment to the national polio laboratory network for poliovirus detection and identification. Two stool specimens were collected for virological diagnosis of poliovirus infection. To be considered adequate, the two specimens must have been collected within 14 days of paralysis onset, at least 24 h apart, and must have arrived at the laboratory in good condition (i.e., reverse cold chain maintained; container not leaking; good specimen quality and adequate quantity). All polio laboratories in this network undergo annual accreditation coordinated by the World Health Organization (WHO). The clinical and laboratory data were maintained in a database at the National Polio Surveillance Unit in New Delhi.

Laboratory methods

Standard procedures were used to isolate viruses from stool suspensions by culture in a rhabdomyosarcoma (RD) and HEp-2C cell monolayer [5]. HEp-2C cells were replaced by L20B cells by the second half of 1999. Serotypes were determined by neutralization tests using high-titre poliovirus equine antisera. Poliovirus isolates were further characterized as Sabin vaccine-like or wild by hybridization with genotypic probes [6], enzyme-linked immunosorbent assay [7], and polymerase chain reaction analyses [8].

Statistical methods

Specimen sensitivity for poliovirus isolation was calculated using the method described by Gary et al. [9], assuming independence of the two specimens. Details are provided in the Appendix. Data are presented as means $\pm$ S.D. Exact binomial 95% confidence intervals were calculated for the individual specimen sensitivities. The formula for 95% confidence intervals for person sensitivity is given in the Appendix.

RESULTS

From 1998 to 2000, timeliness, completeness and quality of stool specimen collection from AFP cases in India improved. The proportion of cases with two adequate specimens increased from 57% to 80% during this period. In 2000, at least one stool specimen

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Table 1. *Characteristics of acute flaccid paralysis (AFP) cases reported in India, 1998–2000*

	1998	1999	2000
All cases	9466	9587	8104
At least one stool specimen (% of all cases)	8069 (85%)	8881 (93%)	7827 (97%)
Median (range) time interval (days) between onset of paralysis and first specimen	8 (0–466)	7 (0–184)	7 (0–88)
Proportion with adequate specimen	6052 (64%)	7192 (75%)	6820 (84%)
Cases with wild poliovirus	1934	1126	265
Cases with wild poliovirus type 1 only (% of cases with wild virus)	1665 (86%)	385 (34%)	138 (52%)
Cases with wild poliovirus type 3 only (% of cases with wild virus)	158 (8%)	718 (64%)	126 (48%)
Cases with both wild poliovirus type 1 and 3 (% of cases with wild virus)	28 (1%)	12 (1%)	1 (0·4%)
Two stool specimens (% of all cases)	7678 (81%)	8682 (91%)	7717 (95%)
Two adequate stool specimens (% of all cases)	5410 (57%)	6743 (70%)	6512 (80%)

Table 2. *Number of poliovirus isolates from first, second or both specimens from acute flaccid paralysis (AFP) cases with two adequate stool specimens, estimated sensitivity of both specimens separately and combined – India, 1998–2000*

	1998	1999	2000	Total*
Specimen 1+, Specimen 2+	869	634	178	1681
Specimen 1+, Specimen 2−	282	171	30	483
Specimen 1−, Specimen 2+	262	122	24	408
Estimated total cases	1498	960	236	2689
Sensitivity of specimen 1 (95% CI)	0·77 (0·74–0·79)	0·84 (0·81–0·86)	0·88 (0·83–0·92)	0·81 (0·79–0·82)
Sensitivity of specimen 2 (95% CI)	0·76 (0·73–0·78)	0·79 (0·76–0·82)	0·86 (0·80–0·90)	0·78 (0·76–0·79)
Combined sensitivity of both specimens (95% CI)	0·94 (0·93–0·95)	0·97 (0·96–0·97)	0·98 (0·97–0·99)	0·96 (0·95–0·96)

* See Appendix for calculation of total.

was collected from 97% of all reported AFP cases within a median of 7 days after paralysis onset (Table 1). For cases with two adequate stool specimens, the first specimen also was collected within a median of 7 days. Among all AFP cases with two specimens, the amount of time between the two specimens ranged from 1 to 61 days, with 98% of the specimens collected 1–10 days apart.

Table 2 shows the overall specimen sensitivity (1998–2000) for the first stool specimen was 81% and for the second stool specimen it was 78%. Person sensitivity for two specimens was 96%. The specimen sensitivities of both specimens increased from 1998 to 2000. From 1998 to 2000, the specimen sensitivity of specimen one increased from 77% to 88% and the specimen sensitivity of specimen two increased from 76% to 86%. The combined specimen sensitivity of poliovirus isolation from two specimens increased from 94% to 98% in the same time period.

When stool specimens were adequate, sensitivity did not differ much depending on when the specimen was taken. First specimens that were taken in the first week after paralysis onset had slightly higher specimen sensitivity than first specimens taken during the second week after paralysis onset (82% *vs.* 78%, respectively) (Table 3). The specimen sensitivity for the second specimen was also slightly higher if the first specimen had been collected in the first week (79% *vs.* 76%). The sensitivity of both specimens combined was 96% if the first specimen was collected in the first week and 95% if the first specimen was collected in the second week. Similarly, there was little difference in specimen sensitivity for poliovirus type 1 and poliovirus type 3. Both specimens individually were more

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Table 3. *Sensitivity of first and second poliovirus specimens from acute flaccid paralysis (AFP) cases with two adequate specimens, stratified by time of first specimen and by poliovirus type – India, 1998–2000*

	n	Specimen 1	Specimen 2	Combined
First specimen during week 1 (95% CI)	11 875	0·82 (0·80–0·84)	0·79 (0·76–0·81)	0·96 (0·95–0·97)
First specimen during week 2 (95% CI)	8265	0·78 (0·75–0·81)	0·76 (0·73–0·79)	0·95 (0·94–0·95)
Wild poliovirus type 1 only (95% CI)	1966	0·78 (0·75–0·80)	0·76 (0·73–0·78)	0·95 (0·94–0·95)
Wild poliovirus type 3 only (95% CI)	858	0·86 (0·83–0·88)	0·81 (0·78–0·84)	0·97 (0·96–0·98)

Table 4. *Number of wild poliovirus-positive first and second stool specimens from acute flaccid paralysis (AFP) cases with two specimens – India, 1998–2000*

	1st specimen positive	Only 2nd specimen positive	Proportion of cases identified only by second specimen (%)
1998	1470	379	20
1999	949	159	14
2000	228	31	12
First specimen during week 1	1424	250	15
First specimen during week 2	864	200	19
First specimen during week 3	185	44	19
First specimen after week 3	174	75	30
Poliovirus type 1 only	1686	418	20
Poliovirus type 3 only	847	133	14
Cases with 2 specimens			
Both specimens adequate	2164	408	16
1st specimen adequate, 2nd specimen inadequate	104	36	26
1st specimen inadequate, 2nd specimen adequate	3	1	25
Both specimens inadequate	376	124	25

sensitive for poliovirus type 3. The combined sensitivity for both specimens showed very little difference for type 3 and type 1 (97% *vs.* 95%, respectively).

Overall, from 1998 through 2000, collection of a second specimen increased sensitivity for poliovirus detection by 15% among AFP cases with two adequate stool specimens. The added sensitivity from a second specimen declined from 18% in 1998 to 10% in 2000 (Table 2). When the first specimen was collected in the first week after paralysis onset, the second specimen increased sensitivity by 14%. For first specimens collected in the second week, the collection of a second specimen increased specimen sensitivity by 17%. The increase in sensitivity from a second specimen was larger for poliovirus type 1 (17%) than for poliovirus type 3 (12%) (Table 3).

Examining all cases with two stool specimens, regardless of whether both specimens were adequate, we determined the proportion of cases identified as poliomyelitis based on only the second specimen (Table 4). The proportion of confirmed poliomyelitis cases that would have been missed if the second sample had not been taken decreased from 21% in 1998 to 12% in 2000. The second sample detected a larger proportion of cases as the interval between paralysis onset and collection of the first stool specimen increased. Among AFP cases whose first stool was collected 3 or more weeks after paralysis onset, 30% of

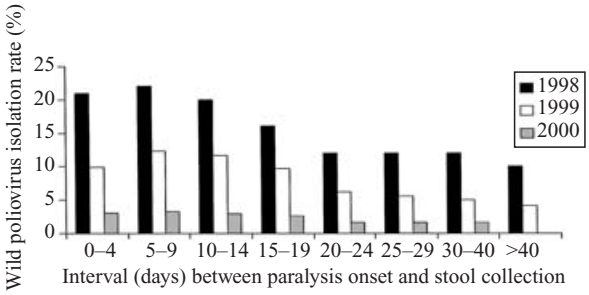

Fig. 1. Wild poliovirus isolation rate, all stool samples, all acute flaccid paralysis (AFP) cases, India, 1998–2000.

the confirmed cases were detected by the second specimen alone. Among all AFP cases, the proportion of poliomyelitis cases identified by only the second specimen was higher for poliovirus type 1 than for poliovirus type 3 (20% *vs.* 14%, respectively). The second specimen also contributed more to poliovirus detection among AFP cases with one or two inadequate stool specimens (25–26%) than among cases with two adequate stool specimens (16%). We found no difference in the proportion of cases identified only by the second specimen for APF cases with the two specimens $\leqslant$48 h apart compared to those with the two specimens $>$48 h apart (17·7% *vs.* 18·1%, respectively).

Figure 1 shows the proportion of all stool specimens collected each year that were found to contain wild poliovirus, by the number of days after paralysis onset. While poliovirus isolation rates were highest (12% overall) among stool specimens collected less than 10 days after paralysis onset, poliovirus was still isolated in 8% of the specimens collected 20–40 days after onset.

DISCUSSION

The India AFP surveillance system provided a unique opportunity to examine the evolution of stool specimen sensitivity during a time when wild poliovirus transmission has been rapidly declining. The high quality of surveillance data and the large number of AFP cases and stool specimens collected allowed us to examine the relationship of stool specimen sensitivity to several variables. As eradication progresses and wild poliovirus circulation becomes confined to areas with difficult access and limited infrastructure, it will be increasingly important to increase confidence that wild virus circulation is not being missed.

As the surveillance system improved from 1998 to 2000, the percentage of reported AFP cases with two adequate stool specimens collected increased. At the same time, specimen sensitivity for the detection of wild poliovirus increased. This reflects a concerted effort to ensure timely collection of two stools after case notification, appropriate storage and transportation of specimens from the field to regional laboratories, and good, consistent laboratory techniques. Another key reason for the increasing proportion of AFP cases with two adequate stool specimens was the expansion of the network of surveillance medical officers (SMOs), which allowed each SMO to be responsible for overseeing fewer districts [4]. Additional factors that contributed to adequate stool collection in India were improved training of district health workers, improved collaboration between SMOs and district health workers who collect and transport specimens, and fewer delays in reporting AFP cases to the surveillance system so that both specimens could be collected within two weeks of paralysis onset.

Among AFP cases with two adequate stool specimens, the sensitivity of the first specimen increased from 77% in 1998 to 88% in 2000. Since the median time interval between paralysis onset and collection of the first stool remained fairly constant from 1998–2000, this increased sensitivity is unlikely to be solely a result of improved timeliness of the first stool specimen. Other factors that might be related to increased sensitivity over this time period are improved collection, storage and transport of specimens, as well as improved laboratory performance, though India laboratory performance was judged adequately high throughout this time period. None of these factors were evaluated in this study.

For each year, sensitivity of the second specimen was lower than for the first. However, sensitivity of the second specimen also increased from 76% in 1998 to 86% in 2000. The added sensitivity from the second specimen declined over time but in 2000 the second specimen still increased sensitivity by about 10%. The diminishing contribution of the second specimen is related to the fact that sensitivity from the first specimen increased during this time and therefore there was less for the second specimen to add.

An earlier analysis by Pinheiro and colleagues used data from the Pan American Regional Poliomyelitis Laboratory network to examine surveillance sensitivity [10]. Analysis of 76 AFP cases from 1987 to 1989 with two stool specimens showed that the wild poliovirus isolation rate from the second sample was 8%. This was calculated as the proportion of cases where the first sample was negative and the second

sample was positive. Because the analysis is based only on patients who were identified as infected with wild poliovirus, it does not account for results that were negative on both specimens, in contrast to the method by Gary et al. used in this analysis [9]. None of the cases identified by the second sample identified new areas of wild poliovirus transmission; thus, programmatically, collection of a second specimen did not offset the substantial laboratory workload required to process a second stool specimen from every AFP case. The authors suggested that in polio-endemic countries with laboratories performing at high levels of proficiency, collection of a second stool from every AFP case might not be necessary. Nevertheless, at this stage in the global eradication effort, even relatively small gains in sensitivity from collecting a second stool may assist in both identifying areas with ongoing transmission of wild poliovirus and providing assurance that areas with ongoing transmission are not being missed. This is particularly true in countries where the AFP surveillance system does not function optimally (e.g. challenges in specimen collection, transport, or cold chain deficiencies). In these countries, the gain of 10–20% in sensitivity that is added by the second specimen may make a difference in the ability to detect areas of ongoing polio transmission, and the collection of two specimens remains 'best practice'.

A limitation of our analysis is that the calculations for the combined sensitivity of both specimens require the assumption of independence for the sensitivity of each specimen. In practice, this assumption is frequently not met. Typically, the same health worker collects both stool specimens from each AFP case. The first is stored 24–48 h until the second is collected, and then both are transported together to the laboratory. Factors affecting specimen quality (e.g. handling by the transporter, packaging, heat) are likely to affect both specimens equally. Our assessment of specimen sensitivity essentially measures the sensitivity of poliovirus detection given that viable virus arrived in the laboratory. A second limitation is that midway through 1999 Indian laboratories began using L20B cells rather than HEp-2C cells for poliovirus isolation. L20B cells increase specificity and decrease sensitivity compared to HEp-2C cells [11], complicating comparisons of specimen sensitivity from 1998 to 2000.

It is programmatically important to identify and correct factors that lead to the collection of too few or inadequate stools. Persons who become very ill or die shortly after paralysis onset are unlikely to provide two stool specimens. Among cases with two stool specimens collected, late specimen collection is the primary cause of inadequate stool collection. In a very few cases, specimens do not arrive at the laboratory in good condition. Delayed stool specimen collection may result from delays in case notification (a shortcoming of the reporting network). Identifying reasons for failing to collect two adequate stools is important so that steps can be taken to minimize these failures of the AFP surveillance system and increase confidence in the ability to detect any circulating wild poliovirus.

Analysis of all stools collected from AFP cases from 1998 to 2000 showed that wild poliovirus was isolated from a small but not insignificant number of stool specimens collected more than 2 weeks after paralysis onset. This corresponds to the findings of Alexander and colleagues, who reviewed several studies of poliovirus excretion and showed that excretion lasts for 3–4 weeks in a high proportion of cases and may continue for 5–6 weeks [12]. In India in 2000, wild poliovirus was isolated from AFP cases as many as 34 days after paralysis onset. The experience in India indicates that the current policy of collecting a stool specimen up to 60 days after paralysis onset is justified and may contribute to the ability of the surveillance system to detect areas of wild poliovirus circulation. Collection of a second stool specimen, even after the 2-week window for 'adequate' stool collection, is particularly useful among AFP cases where the first stool specimen was collected late. During the early phase of the disease, poliovirus excretion is consistent but it may become intermittent as the immune response of the patient develops over time. Therefore, the value of the second stool specimen is greater when specimen collection is delayed beyond 10 days after paralysis onset.

AFP surveillance systems are used to detect areas with wild poliovirus transmission. Collecting a second specimen can lead to increased sensitivity for detecting an AFP case with wild poliovirus infection and may increase the possibility of uncovering previously undetected areas of poliovirus transmission. Thus, it is crucial that all efforts be made to collect two stool specimens from every AFP case, even if the two specimens cannot be collected within 14 days of paralysis onset.

The experience gained from India's AFP surveillance system can be applied to other countries as eradication nears. In the final stage of eradication, wild poliovirus transmission will likely be limited to

countries with poor infrastructure and AFP surveillance systems functioning at sub-optimal levels. In these situations, it will be prudent to collect a second stool specimen for all AFP cases. Once wild poliovirus transmission has not been documented for 3 years with adequate surveillance, certification of a polio-free world will require assurance that circulating wild poliovirus has not been missed. High levels of adequate stool specimen collection from all AFP cases will be crucial to achieve adequate sensitivity to provide this assurance.

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APPENDIX

The estimators for unequal specimen sensitivities ($\hat{\gamma}_1$ and $\hat{\gamma}_2$) are as follows:

$$\hat{\gamma}_1 = \frac{N_{11}}{N_{11}+N_{01}}$$

and

$$\hat{\gamma}_2 = \frac{N_{11}}{N_{11}+N_{10}},$$

where N_{11} is the number of persons with both specimens positive, N_{10} is the number whose first specimen was positive and the second negative, and N_{01} is the number whose first specimen was negative and the second positive. The number of infected individuals with both specimens negative, N_{00}, is estimated using standard capture–recapture methods as

$$\hat{N}_{00} = \frac{N_{01}N_{10}}{N_{11}}.$$

The estimated total number of cases, $\hat{N}$ (Table 2) is thus $\hat{N} = N_{11}+N_{10}+N_{01}+\hat{N}_{00}$. The combined sensitivity of both specimens, person sensitivity [9], was calculated as:

$$\hat{\gamma} = 1-(1-\hat{\gamma}_1)(1-\hat{\gamma}_2).$$

Confidence limits for person sensitivity

To find exact confidence bounds for the sensitivity to detect an infected person (person sensitivity) using two stool samples, it is convenient to begin with the probability distribution of the number of persons having two positive stools, $N_2 = N_{11}$, given that the person has at least one positive stool. When the specimen sensitivities for each stool are equal, the probability that both are positive given that at least one is positive is

$$\rho = \frac{\phi^2}{1-(1-\phi)^2}.$$

Therefore, N_2 follows a binomial distribution with parameters T and ϕ, where T is the total number of persons with one or more positive stool samples (i.e. $T = N_{11}+N_{10}+N_{01}$). An exact upper or lower bound for ρ can then be found by using any program for finding exact limits for the success probability of a binomial distribution. Once the lower (or upper) bound ρ_L is found, the corresponding lower (or upper) bound for ϕ, ϕ_L, can be found from the relationship

$$\phi_L = \frac{2\rho_L}{1+\rho_L}.$$

Finally, the lower (or upper) limit for person sensitivity is given by

$$\theta_L = 1-(1-\phi_L)^2.$$

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