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Isolation Rate of Enteroviruses vis-a-vis Number of Samples and Mode of Collection

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Acute paralytic poliomyelitis is essentially a clinical diagnosis. The clinical diagnosis in a large number of cases of paralytic poliomyelitis is not confirmed by laboratory investigations. Absence of virology laboratory and a straightforward, clearcut clinical presentation are the main reasons for lack of laboratory support.

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*Received for publication: October 8, 1993;
 Accepted: March 9, 1994*

For isolation of polio and other enteroviruses, the commonest clinical material required to be collected is feces. This is obtained in the form of voided stool. In recent years, collection of feces is practised by Anal Tube Technique. It is a considerable improvement over rectal swab technique. Anal tube technique has advantages of simplicity and rapidity especially under "field" conditions.

A study to compare the rate of isolation of viruses from fecal material collected by two methods namely Voided Stool Sample Technique (VSST) and Anal Tube Technique (ATT) was undertaken on cases of paralytic poliomyelitis.

Material and Methods

Anal tube (AT) is a thick hollow glass tube of 125 mm in length and 0.5 mm in diameter with smoothed edges. The sterile AT was lubricated with sterile glycerine and gently introduced in the anal canal up to 20 to 30 mm, rotated and was gradually withdrawn. The AT was then placed inside a stoppered tube containing 3 ml of minimum essential medium. Voided stool sample was collected in a sterile container. From 116 clinical cases of paralytic poliomyelitis, multiple samples (2 to 7) were obtained by

both VSST and ATT methods in the acute phase of illness. The average interval between the onset of paralysis and the collection of the first sample was 3.4 days. These multiple samples were obtained in a span of maximum 15 days. The details of age group of 116 cases were as follows: Up to 1 year 45 cases (38.8%), between 1 to 2 years 47 cases (40.5%), between 2 to 3 years 19 cases (16.4%) and age greater than 3 years 5 cases (4.3%). All these samples were processed and inoculated independently for isolation of virus.

Ten per cent suspension of fecal material obtained by VSST method and in the case of ATT method medium in the test tube (after discarding the anal tube) was centrifuged in a cold centrifuge at 3000 RPM for one hour. The supernatant was removed and an equal amount of ether was added to it and it was kept at 5 to 7° C overnight. Ether was removed and 5000 IU of penicillin and 10 mg of streptomycin per ml of fecal suspension were added, pH was adjusted to 7.2.

Isolation of virus was attempted on monolayer of Vero cells grown in tissue culture tubes. Minimum essential medium with 2% of sheep serum was used for maintenance of Vero cells. Processed fecal sample (0.3 ml) was inoculated on three tissue culture tubes each. The cells were observed for 7 days for cytopathic effect (CPE). Cells showing CPE were frozen and the thawed fluid was taken up for identification of virus. Fluid from the tubes showing no CPE on primary inoculation was "harvested" and reinoculated into three fresh tissue culture tubes (blind passage).

Identification of virus was carried out by the neutralization test on Vero cells using all the three types of specific poliovirus antisera.

Any one of the multiple samples showing presence of polio or non-polio enterovirus was considered positive for virus isolation by that method and for that patient. By this approach, rate of isolation of virus by the two methods of collection for the first sample, for first 3 and for first 7 samples were compared. The two methods of collection of samples (ATT and VSST) were comparable as these two methods were employed on the same patients.

Results

All the three polio virus types and non-polio viruses were isolated by both VSST and ATT methods. The patient-wise distribution of viruses was as follows: poliovirus type 1-19 (VSST) and 17 (ATT); poliovirus type 2-22 (VSST) and 14 (ATT); poliovirus type 3-34 (VSST) and 30 (ATT); non-polio viruses-6 (VSST) and 7 (ATT); mixture of polio viruses-2 (VSST) and 2 (ATT); mixture of polio and non-polio viruses—9 (VSST) and 11 (ATT); and negative for virus isolation 24 (VSST) and 45 (ATT).

Results of this study are presented in *Table I*. It was observed that the rate of isolation of virus varied considerably from 39.6% (one sample by ATT method) to 81.1% (7 samples by VSST method). Higher rates of isolation of virus were obtained by VSST method than by ATT method. The differences in the rate of isolation of virus by two methods at '7 sample', '3 sample' and '1 sample' levels was statistically significant and the p values were $p < 0.05$ (chi square value 4.42), $p < 0.01$ (chi square value 10.18), and $p < 0.01$ (chi square value 7.60), respectively.

Multiple samples in the same method gave higher rate of isolation. By VSST method, the rate varied from 57.7% (1 sample) to 81.1% (7 samples). In the case of

TABLE I—Results of Isolation of Enteroviruses by two Methods of Collection of Fecal Material

Method	Results of isolation of enteroviruses		
	7 samples	3 samples	First sample
VSST**	43/53*	80/106	67/116
Per cent positive	81.1	75.4	57.7
ATT**	46/72	62/113	46/116
Per cent positive	63.8	54.8	39.6

* Numerator = Number of cases positive for enterovirus isolation.

* Denominator = Number of cases studied for enterovirus isolation.

** VSST-Voided Stool Sample Technique.

** ATT-Anal Tube Technique.

ATT method, the rate varied from 39.6% (1 sample) to 63.8% (7 samples).

Discussion

Ideally, every clinical diagnosis of paralytic poliomyelitis should be supported by virological investigations. This is especially essential in the present situation in India as declining trends in incidence have set in(1). Therefore, reporting of every case of acute flaccid paralysis is important and the case should be virologically investigated. It is necessary to employ more sensitive methods for collection of fecal material. There is every likelihood of obtaining a false negative result if a less sensitive method is used as shown in our study.

The study also shows the importance of obtaining multiple samples whatever method is employed.

Acknowledgements

The authors gratefully acknowledge the help and critical comments offered by Dr. F.M. Rodrigues, Scientist Emeritus, National AIDS Research Institute, Pune in preparing this write up. Grateful thanks are also due to Dr. (Mrs) JJ. Rodrigues, Director, Enterovirus Research Centre, Bombay for encouragement and constructive suggestions.

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