JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 1981, p. 388-389
0095-1137/81/020388-02$02.00/0

Vol. 13, No. 2

NOTES

Human Infective Dose Determinations for Oral Poliovirus Type 1 Vaccine in Infants

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The 50, 10, and 1% human infective doses of poliovirus type 1 vaccine administered orally to 32 infants were estimated to be 72, 39, and 20 tissue culture infective doses, respectively.

Exposure to waterborne viruses may occur through consumption of contaminated water, shellfish, or other foods in contact with contaminated water, or as a result of recreational activities involving water. Consequently, attempts have been made to estimate the levels of contamination that pose significant hazards to human health. One approach has been to determine the 50% tissue culture infective doses ($TCID_{50}$) or plaque-forming units of attenuated poliovirus strains that constitutes a minimum human infective dose (HID).

Early studies by Koprowski (4, 5) with type 1 (SM strain) poliovirus administered in hard gelatin capsules reported that two of three children were infected with a dose of 2 plaque-forming units, whereas infection did not occur in two subjects who received 0.2 plaque-forming units. Katz and Plotkin (3) administered type 3 (Fox strain) poliovirus by gavage tube to 22 premature infants. They calculated that the 50% HID (HID_{50}) and HID_{10} were equivalent to 4 $TCID_{50}$ and 0.3 $TCID_{50}$, respectively. These data led Berg (1) to conclude that "any amount of virus in drinking or recreational water that is detectable in appropriate cell cultures constitutes a hazard to those drinking the water."

Delivery of virus doses directly to the gastrointestinal tract, as in the above-cited studies, may result in an underestimation of the oral HID. A consistently higher HID was reported when doses were delivered to the oral cavity (6, 9, 10). Plotkin et al. (9) reported that oral doses of $10^{1.5}$ to $10^{1.9}$ $TCID_{50}$ of type 3 (Fox strain) poliovirus infected seven of nine infants, whereas the HID_{50} of type 1 (CHAT strain) was $10^{4.0}$ to $10^{4.9}$ $TCID_{50}$.

The present study was done to provide additional data on the HID of orally administered attenuated polioviruses. Doses of 7 to 280 $TCID_{50}$ of live, oral poliovirus type 1 vaccine (lot 262-I, postfiltration sample, Pfizer Ltd., Sandwich, Kent, England) were administered to 32 2-month-old infants.

Doses were prepared by making a suitable series of dilutions of vaccine in sterile pharmaceutical water (water for injection, USP, Invenex Pharmaceuticals, Chagrin Falls, Ohio). The viral infectivity titer of each dose was determined with the tube method (7), using eight replicates per dilution, Wisl cell cultures (a human embryonic lung diploid strain developed by the Wisconsin State Laboratory of Hygiene Virus Section), and incubation at 37°C for 7 days.

Infants, patients of a private pediatric practice, were recruited before the time that they were scheduled for the first dose of trivalent oral poliovirus vaccine. Each dose consisted of 0.5 ml of final vaccine dilution which was delivered into the oral cavity of the infant with a 1-ml syringe. Each infant was carefully observed to be certain that the dose was not expectorated.

A stool sample or rectal swab was collected at the time vaccine was administered. Stool specimens were collected daily thereafter for 10 days. Stools were processed by emulsifying a 2-g sample in 8 ml of a 0.03% aqueous solution of $NaHCO_3$ containing antibiotics. The suspension was centrifuged at 13,000 rpm (14,000 × *g*) for 30 min in a Spinco model L ultracentrifuge. Eight tubes of Wisl cell culture were each inoculated with 0.2 ml of supernatant fluid. The tubes were incubated in a stationary position at 37°C for 2 weeks and examined periodically for cytopathic effect. Suspected isolates were passed once.

The first and last isolates obtained during the postfeeding period were tested for neutralization by a rabbit hyperimmune antiserum to poliovirus type 1 (Microbiological Associates, Be-

TABLE 1. *Response of 32 infants to oral doses of poliovirus type 1 live vaccine*

Prefeeding antibody titer	No. infected/no. tested at following $TCID_{50}$:											
	280	210	160	90	80	65	55	50	42	27	16	7
<1:8				1/2		0/3	1/1	2/4			0/1	
≥1:8		2/2	3/3	2/2	1/1	0/3	0/2		0/1		0/1	
No serum	1/1							1/2		0/2		0/1

TABLE 2. *Estimation of the $TCID_{50}$ equivalent to selected HID*

% HID	Estimated $TCID_{50}$	95% Confidence interval[a]
50	72	55–93
10	55	24–63
1	20	7–52

[a] Lower and upper limits of the $TCID_{50}$.

thesda, Md.). A 50-μl volume of cell culture growth medium containing 32 to 320 $TCID_{50}$ of virus was mixed with 50 μl of medium containing 20 U of antiserum in two wells of a microtiter plate and incubated at 37°C for 1 h in a 5% CO_2 atmosphere. A 0.15-ml volume of a suspension of Wisl cells in growth medium was added to each well. The plate was reincubated for 6 days and examined for a viral cytopathic effect. Available infant sera were tested for neutralizing antibody to the type 1 vaccine strain with a microtiter procedure (8) and Wisl cell cultures. The criterion for establishing an infection was isolation of poliovirus type 1 from a stool sample.

The influence of detectable prefeeding poliovirus type 1 antibody on the estimated HID values was difficult to evaluate because of the small number of infants in the study. Nevertheless, the presence of maternal antibodies did not appear to be a major factor. The HID_{50} for those with preexisting antibody was between 65 and 80 $TCID_{50}$, whereas the value for those lacking antibody was between 50 and 90 $TICD_{30}$ (Table 1). Statistical analysis, by the logit method developed by Berkson (2), estimated an HID_{50} of 72 $TCID_{50}$ based on the data for all 32 infants (Table 2). Estimates for the HID_{10} and HID_1 are included in Table 2, but caution is necessary in interpreting these estimates since relatively few children were given doses at the lower ranges.

The results of this study confirm previous findings (6, 9, 10); i.e., the HID_{50} of attenuated polioviruses is not equivalent to the $TCID_{50}$ when doses are delivered to the oral cavity as opposed to direct delivery to the gastrointestinal tract. These findings are reasonable, for there must be a considerable loss of virus in the oral cavity and during passage through the alimentary tract. However, extrapolation of these data to natural situations may not be valid. In addition to the distinct possibility that the minimum HID of some viruses found in nature may be much lower, viruses in polluted waters may be protected by substances such as proteins that might allow small amounts of virus to reach the intestinal tract with very little loss.

This work was supported in part by U.S. Environmental Protection Agency grant R-804161.

We greatly appreciate the cooperation of Pfizer Ltd. for supplying the monovalent vaccine used in this study. We thank the staff of the Pediatrics Department of the Dean Clinic, Madison, and their patients for their cooperation. Technical assistance was provided by S. Gould, P. Parks, and C. Westphal.

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