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Project Summary

Determination of Minimal Infectious Dose of an Enterovirus in Drinking Water

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The goals of this project were to determine the minimal infectious dose and the medical significance of an enteric virus (Echovirus-12) ingested in drinking water. The study was conducted under double-blind, placebo-controlled, random-selection conditions. A total of 149 susceptible (antibody-free), healthy, young adult males were placed in isolation and ingested varying amounts of Echovirus-12 (10-10,000 pfu) or placebo seeded into 100 ml of nonchlorinated water. The subjects were followed for evidence of illness, pharyngeal and intestinal (fecal) viral shedding, and serum antibody response.

No illness or evidence of infection occurred among the placebo subjects. Infection in exposed subjects was primarily based on fecal shedding of virus. Only four subjects experienced a significant seroconversion in the absence of demonstrable fecal viral shedding. Similarly, pharyngeal shedding of virus without evidence for fecal viral shedding was sporadic and was observed in only five subjects. Fecal shedding and/or significant seroconversion occurred in 30% of the subjects given 10 pfu (the lowest dose administered) and in 100% of the 12 subjects given 300 pfu as measured in a plaque assay using LLC-MK_2 cells. Fecal shedding persisted for as long as four weeks in some subjects.

***Statistically, it was inferred from* probit analysis that ingestion of one to two pfu of Echovirus-12 would infect 1% of the population.**

This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

Background

Waterborne outbreaks of viral disease continue to occur. Viruses which are known or highly suspected to contaminate untreated and/or treated waters are hepatitis A virus (now tentatively classified as an enterovirus), enteroviruses, adenoviruses, and the group of nonbacterial gastroenteritis viruses (rotaviruses, Norwalk agent, and others). Current technology is capable of detection of small quantities of viruses in relatively large amounts of water. Because enteroviruses are known to occasionally survive water treatment, they are a potential hazard to human health. What is not known is the medical significance of small amounts of viral contamination which may occur in potable waters.

For economic and scientific reasons, there is no widespread program for monitoring viruses in drinking water, and very little data are available about health effects of low-level viral contamination of water supplies. This project was undertaken to demonstrate the critical level of water contamination which produces an infection of illness in humans. The results would provide a basis for more informed decisions regarding the establishment of standards for viral content of treated waters.

To determine this critical level, experimental viral "challenge" studies were conducted under rigidly controlled conditions.

Susceptible subjects were chosen, known doses of a human virus were administered at recorded times, and subjects were carefully observed and monitored using laboratory sampling procedures. The research team used a viral representative found in water, and administered this virus to subjects in drinking water. During its use in a previous experimental viral challenge study, this virus had not been associated with serious human illness.

Specific Goals

The specific goals of the study were to determine in susceptible, healthy, adult subjects: (1) the minimal infectious dose of Echovirus-12 ingested in drinking water and (2) the medical significance of small amounts of an enteric virus consumed in this manner.

Study Design

The study was designed to select healthy, young, adult male subjects who lacked detectable serum hemagglutination-inhibition (HI) antibodies to Echovirus-12, place them in isolation, inoculate them with varying doses of the virus (10-10,000 pfu assayed in LLC-MK_2 cells) or placebo (tissue culture fluid) and follow them for evidence of illness, viral shedding in the pharynx and intestinal tract, and production of serum antibody. The assignment of inoculum (viral dose or placebo) was done by random selection, and all clinical and laboratory assays were performed under double-blind, placebo-controlled conditions. Viral detection and identification were done in LLC-MK_2 tissue culture tubes with cytopathic effect as an endpoint. Antibody assays were done by a "standard" hemagglutination-inhibition test and by a microtiter neutralization test using LLC-MK_2 cells.

Results and Discussions

Six separate studies were conducted using a total of 189 subjects. Forty subjects participated in Study 1, in which tap water at the isolation site was used as the vehicle for inoculation. About 45 minutes prior to ingestion by the subjects, the virus doses were suspended in tap water, having a free residual chlorine of 0.8 mg/l. Subsequent tests indicated that 100 pfu of Echovirus-12 suspended in this tap water was reduced to a non-detectable level within 45 minutes when assayed in LLC MK_2 cell culture. At the dosage levels administered in experiment 1, subsequent feeding experiments indicated that 18 of the subjects administered virus would likely have become infected if the inoculum had been fully viable. However, none of the 30 subjects who ingested virus suspended in chlorinated tap water became ill or yielded evidence of infection. Chilled distilled Talawanda water* was used as a vehicle in the remaining five studies.

Study 2 was a range-finding study, which did not include placebo controls. Viral doses ranged from 10-10,000 pfu. Dosages in subsequent studies ranged from 10-300 pfu.

The following results are based on the 149 subjects who participated in studies 2-6. There was no evidence of infection or illness among the placebo subjects. No illness occurred among any virus-inoculated subjects. However, a "dose-response" curve for infection was established. Evidence for infection was based primarily on isolation of Echovirus-12 from fecal swabs. Only four subjects experienced significant antibody responses in the absence of intestinal viral shedding. Likewise, only five subjects were found to shed virus in the pharynx who were not found to be shedding virus in the intestinal tract and in these, virus was found on only one day in each case. The majority of intestinal viral shedding occurred during the first week after administration of viral inocula and persisted for several weeks in some subjects, regardless of the dose.

Using the definition of infection as recovery of virus from fecal specimens and/or a significant serological response, the infection rates were as follows:

Dose (pfu)	No. subjects	No. infected	%
0	34	0	(0)
10	50	15	(30)
30	20	9	(45)
100	26	19	(73)
300	12	12	(100)
1,000	4	2	(50)
10,000	3	2	(67)

Using a probit analysis based on the values presented in the above data for four dosages (10, 30, 100, 300 pfu) it can be inferred, within 95% fiducial limits, that 1-2 pfu would infect 1% of a susceptible population.

Because of the implications of the results found in this study in regard to public health practices and policies, the following points must be considered carefully:

1. The virus used in the study was "safe" and not associated with serious natural disease. Additional studies using other enteric viruses should be conducted.
2. An "end point" (a dose which did not cause infection) was not determined. Accurate delivery of a dose less than 10 pfu is not statistically sound unless very large numbers of volunteers are studied. Therefore, statistical inference was required to determine a minimal infectious dose.
3. "Susceptibility" was defined as no detectable serum antibody at a 1:5 dilution of serum by HI and/or dilution by microtiter neutralization. No effort was made to determine pre-existing intestinal antibody.
4. Only one cell culture assay system was used to titer the viral inocula.

*Mention of trade names or commercial products does not constitute endorsement or recommendation for use by the U S Environmental Protection Agency.

Conclusions

1. No clinical illness occurred following administration of 10-10,000 pfu of Echovirus-12.
2. Infection was detected in 30% of the subjects given 10 pfu indicating that the minimum infectious dose may be considerably lower.
3. Using statistical analysis and extrapolation, it was determined that 1-2 pfu may be infectious for 1% of the population.
4. Intestinal shedding at doses as low as 10 pfu may persist for at least 19 days. At higher doses, the persistence may be longer.
5. Secondary spread was not detected among susceptible adult contacts.
6. Virus ingested in drinking water may sometimes be recovered from the pharynx.
7. Consistent significant serum antibody responses probably require a greater dose (< 10,000 pfu) or a different route of administration.
8. Chlorine-inactivated virus ingested at a concentration up to 100 pfu did not yield a detectable infectious or immune response.

Recommendations

1. Similar studies should be conducted using different viral inocula that might have greater medical significance (yet still be safe), e.g., other enteroviruses rotaviruses.
2. In order to determine the effect of prior infection on susceptibility to low viral doses, the Echovirus-12 study should be extended to include subjects with naturally-acquired serum antibody; those subjects from the current study who received virus and developed serum antibodies, and also those who received and shed virus but did not develop antibodies.

3. Consideration should be given to an investigation of the role of multiple viral ingestions (i.e., more than one viral type at the same time; the same virus several times) and different routes of inoculation, e.g., aerosol.
4. Future investigations should include the collection of pharyngeal washings to further determine the incidence of pharyngeal shedding subsequent to viral ingestion in water.
5. Attempts should be made to determine viral particle content in relation to plaque-forming units in infective dose studies.
6. Evaluation should be made of other cell lines and assay procedures to determine infectivity titers for "challenge" viruses. If more sensitive cell lines are found, additional studies in human subjects should be performed to directly measure viral infectivity at lower doses than used in the present study.
7. Viral shedding should be more accurately quantitated to determine the relationship between dose and viral replication.
8. The relationship between viral infection and local (intestinal) antibody production should be determined, as well as the length of time such antibody is produced.

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***Elmer Akin** is the EPA Project Officer (see below).*
The complete report, entitled "Determination of Minimal Infectious Dose of an Enterovirus in Drinking Water," (Order No. PB 83-191 015; Cost: $8.50, subject to change) will be available only from:
National Technical Information Service
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Telephone: 703-487-4650
The EPA Project Officer can be contacted at:
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