

# Isolation of Poliomyelitis Virus from a Contaminated Well

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**A** PART from infectious hepatitis viruses, there is only theoretical evidence suggesting transmission of viruses by drinking water. Many attempts have been made to implicate drinking water as a means of spreading poliomyelitis, viral gastroenteritis, and diarrhea; however, definite evidence by virus isolation has been lacking. In this report, poliovirus was recovered from water of a contaminated well used by a large restaurant.

Patrons of the restaurant complained to local health authorities on January 13, 1970, that within 30 hours following a meal at the restaurant, they suddenly became ill and were nauseated, vomited, and had diarrhea with prostration. Tests by the Michigan State health laboratories on samples of food, similar to that consumed by the patients, did not contain bacteria commonly associated with foodborne illnesses.

On January 20, 1970, three people had a noon meal at the

restaurant and by 7:30 p.m., one person became ill. Upon questioning, it was learned that the patient had drunk water with her meal but that the other two persons had had another beverage. Again, tests on samples of food eaten by the patient did not contain bacteria that would be associated with foodborne infection.

## Water Source

The restaurant in question is in southeastern Michigan just off a freeway and south of Detroit. The well used by the restaurant is 100 feet deep and the well casing goes 26 feet to limestone where the pump is attached to a 45-foot drop pipe in the casing. The soil composition from the surface is clay the first 18 feet, shale the next 8 feet, and limestone the last 74 feet.

The drainfield behind the restaurant is shown in figure 1. The small houselike structure at the left contains a pump to remove the effluent from the holding

tank. Wastewater is pumped to the drainfield where fluid is evaporated. The well used by the restaurant is more than 300 feet from the edge of the drainfield.

Coliform organisms found in the well water were as follows:

Date	Coliform organisms per 100 ml.
Apr. 1, 1969.....	0
Sept. 25, 1969.....	16 or more
Oct. 2, 1969.....	16 or more
Feb. 24, 1970.....	0
Apr. 16, 1970.....	0

Tests were made by the staff of the State health laboratories.

### Concentration Procedures

Five gallons of water were taken from the well on February 4, 1970. The water was divided into two 2½-gallon samples. Fifty milliliters of water were removed from each sample, and 10 ml. were used as seed for five tubes of lauryl sulfate double strength broth to detect coliform organisms.

The two water samples were then processed identically except that one sample was treated with 60 ppm polyethylene flocculant before centrifugation. Previous tests had shown that flocculant helped remove virus particles from suspension.

Both water samples were centrifuged in a Beckman continuous flow L-4 centrifuge developing 70,000 × gravity. Density gradient was not used in the rotor, and the sediment was concentrated on the bowl surface. The sediment was resuspended in approximately 375 ml. of the remaining water after approximately 375 ml. were removed from the rotor while decelerating.

Two 16 ml. volumes from each of the two samples were removed, and 3 ml. of each sample were used to seed two tubes of 10 ml. each of lauryl sulfate

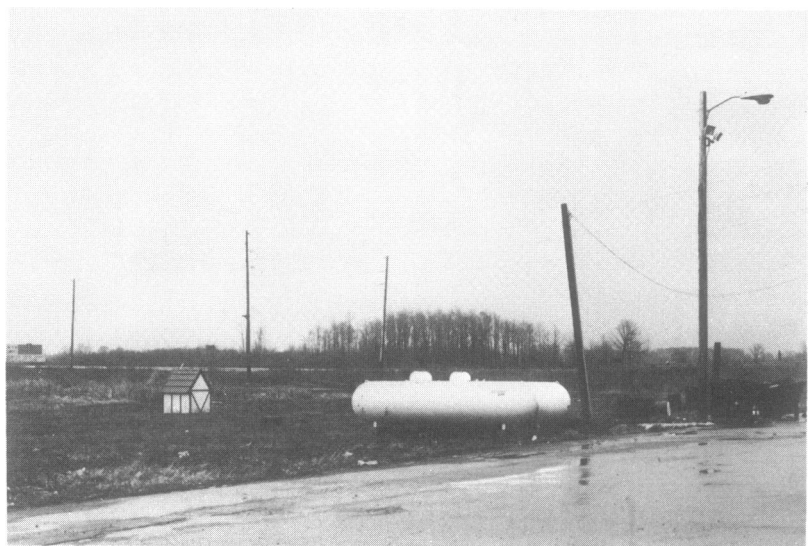


Figure 1. Drainfield behind restaurant

broth to test for coliform organisms. The remaining volumes of water were each subjected to a concentration cycle at 78,480 × gravity in rotor 30.

The pellets from each tube were resuspended into a small amount of the supernatant fluid and pooled. Both concentrated samples were tested for virus on green African monkey kidney cell cultures. A sample of each concentrate was also seeded on appropriate bacteriological medium for the detection of *Salmonella* and *Shigella*.

### Virus and Bacterial Isolations

Virus was recovered only from the sample to which the flocculant was added before concentration. The agent produced a cytopathic effect on the monkey kidney cell cultures and successful passages were made on secondary cultures on human carcinoma epithelial cells.

A second initial recovery was successful from the same concentrated sample. The virus was not sensitive to either, and neutralization tests identified the virus as type II poliovirus. Tissue culture

fluid containing the virus was sent to the Enterovirus Unit of the Center for Disease Control, Atlanta, Ga., where it was determined that the virus recovered was "vaccine-like."

*Escherichia coli* was not recovered in cultures seeded with the two unconcentrated water samples. Cultures seeded with first cycle concentration from both water samples were found to be positive for *E. coli*. No *Salmonella* or *Shigella* were recovered. A diluted sample of the sediment from both water samples was placed on electron microscope screens and examined for viruses.

Many bacteria were present on the screens, and figure 2 shows one of the many organisms seen. Most of the bacteria observed had bacteriophage attached to their surface and resembled organisms observed in preparations made directly from sewage samples. No viruses specific to human beings were recognizable.

On March 10, 1970, a second 5-gallon sample of water was taken from the well. A chlorinator had been installed in the

water system to deliver 2 mg. chlorine per liter of water. The concentration procedure was repeated with this sample but no coliform or viruses were recovered.

## Discussion

Many public health workers believe that the enteroviruses are responsible for many of the episodes of gastroenteritis encountered today, but adequate documentation is lacking.

Mosely (1) lists eight epidemics of poliomyelitis and 50 of infectious hepatitis that were epidemiologically attributed to waterborne transmission of viruses. Viral gastroenteritis episodes are commonly attributed to drinking

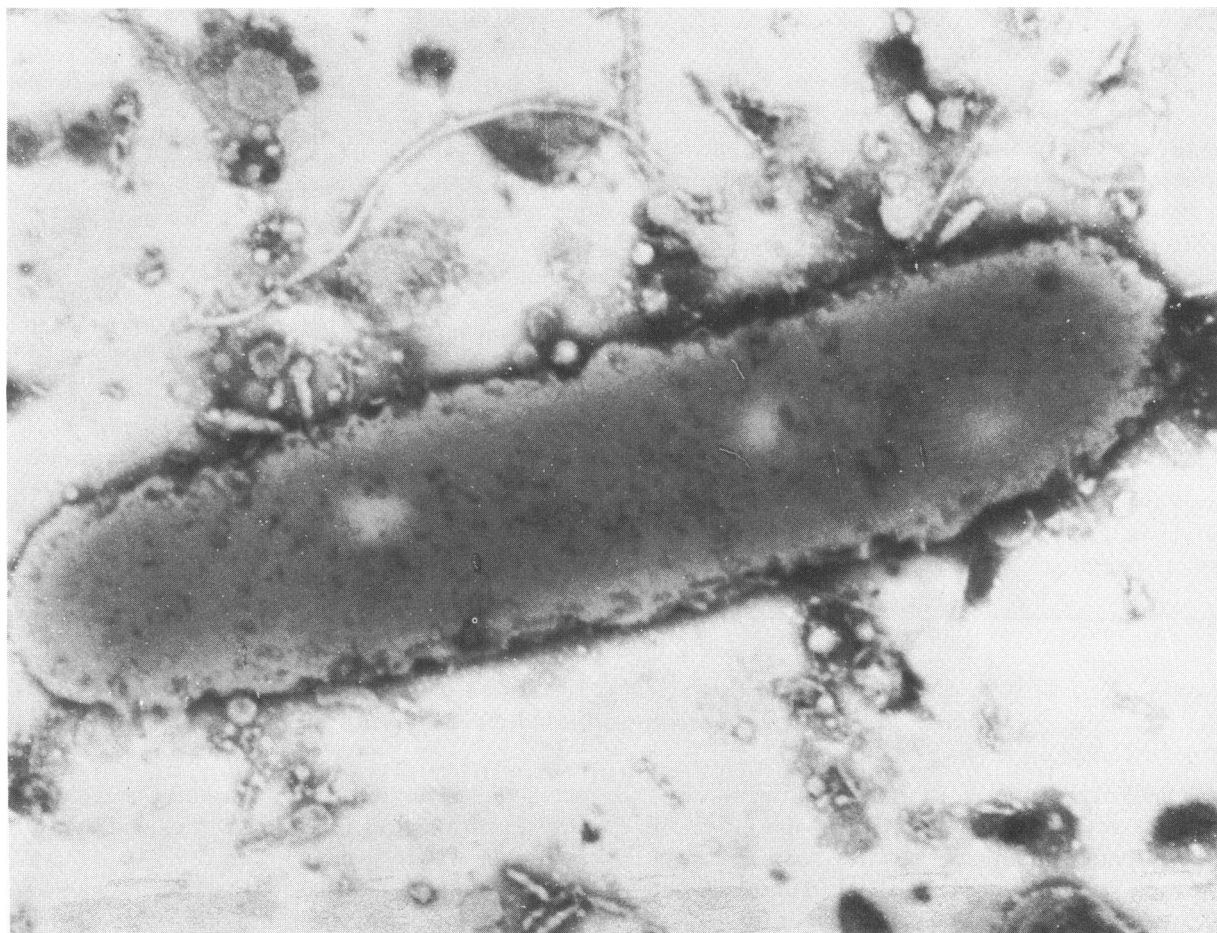
contaminated water. Weibel and co-workers (2) listed 142 cases between 1946 and 1960 in which epidemiologic evidence suggested waterborne infections. Recently Harrel and co-workers (3) reported an incident in which 91 percent of the patients with infectious hepatitis had eaten at a cafe which had contaminated tap-water.

The increased contamination of water sources by an expanding population may cause an increase in pathogens capable of producing waterborne bacteria and viruses which can cause illnesses in human beings. Since bacteria are not always found to be the cause of many cases of gastroenteritis, it may be assumed that the wa-

terborne viruses are responsible for some of the cases of gastroenteritis occurring today.

Our isolation of poliovirus from drinking water from a well is significant. It demonstrates that, with the present methods of concentration and testing, viruses might be recovered when there are outbreaks of gastroenteritis in which no significant bacteria or other parasites are discovered initially.

Patrons of this restaurant were often travelers on the freeway, and they probably would be many miles away from the restaurant before developing symptoms of illness. Occasionally local residents eat at the restaurant, and these would be the only peo-



**Figure 2. Bacterium with attached bacteriophage found in contaminated water (39,270 X)**

ple complaining to the local health department about illnesses following dining at the restaurant. For this reason, the number of people infected by drinking water from this well cannot be determined.

When complaints were received, the State health laboratories suspected bacterial food poisoning and samples of food were tested. No suspicious bacteria were found. The incident on January 20, 1970, was the first suggestion that the drinking water might be the cause of the illness because only the person who drank water with the meal became ill.

The tests showed that the water from the well contained coliform organisms in September and October of 1969. As far as can be determined, nothing was done about the contamination at that time. Coliform organisms were not detected by using un-concentrated water and testing by standard methods on February 4, 1970 (4).

However, when the water sample was concentrated and then tested, coliform organisms were recovered. The organisms were detected only by concentrating the water sample to isolate the viruses. This discovery emphasizes the inadequacy of the coliform test as an indication of water pollution. We emphasize

that both coliform bacteria and virus were recovered only after samples from the supposedly coliform negative water supply were concentrated.

We do not know for certain that the poliovirus isolated from the water was responsible for the illness because we did not attempt to isolate virus from the patient. As sewage was finding its way into the well water, it could have contained any or all of the enteric viruses.

The presence of the Sabin poliovirus vaccine in sewage is understandable. Mass vaccination has resulted in large amounts of "vaccine-like" virus in the sewage. Recently in our laboratory, 93.5 percent of the 77 virus isolates from the local wastewater treatment plant were poliovirus, and there were no known cases of poliomyelitis in the community.

Employing the electron microscope to examine sediments from the water sample after concentration might be valuable in determining biological contamination. Bacteria observed with their infecting viruses were found in this water sample and did not differ from those seen in examining sewage samples with the electron microscope.

The finding of virus in the water from this well emphasizes the need for chlorination of all

water used by the public regardless of the presence or absence of the coliform organism. Whether this would create a situation where the public would then be drinking heavily chlorinated sewage instead of clean water remains to be seen.

After it was determined that the well water contained pathogens, the water system was chlorinated so that bacteria or viruses could no longer be detected. The sewage content of the water remained the same. We feel that the public should not have to drink sewage of any kind in its water.

## REFERENCES

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The water supply of a large restaurant was implicated as a source of infection in the illness of several patrons. Initially, a search for bacteria in the food and water source of the restaurant did not uncover the responsible agent.

However, when 5 gallons of water from the well used by the restaurant were concentrated by flocculation and ultracentrifugation and the sediment

was tested for virus in tissue cell cultures, type II poliovirus was recovered.

The results of the tests emphasized the immediate need to chlorinate all drinking water used by the public and to examine critically methods of handling waste water.