

Recovery of Hepatitis A Virus from a Water Supply Responsible for a Common Source Outbreak of Hepatitis A

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Abstract: An outbreak of hepatitis A occurred in a north Georgia trailer park served by a private well. Of 18 residents who were serosusceptible to hepatitis A virus (HAV), 16 (89%) developed hepatitis A. Well water samples were collected 3 months after illness onset in the index case and 28 days after illness onset in the last trailer

park resident. Hepatitis A virus antigen (HAVAg) was detected in the samples by enzyme immunoassay from three of the five cell lines following two 30-day passages and from a fourth cell line following a third passage of 21 days. (*Am J Public Health* 1990; 80:428-430.)

Introduction

Infection with hepatitis A virus (HAV) accounted for 39 percent of the 59,633 cases of acute viral hepatitis reported in the United States in 1985.¹ Virus transmission occurs primarily through the fecal-oral route, resulting in both sporadic cases and common source outbreaks.² Common source outbreaks of hepatitis A have been associated with ingestion of contaminated food^{3,4} or consumption of inadequately treated drinking water.⁵⁻⁹ The incrimination of food and water in HAV transmission has largely been based on the epidemiologic characteristics of the outbreak and serologic evidence that the resulting viral hepatitis was type A. Only recently has HAV been recovered from water associated with hepatitis A outbreaks.¹⁰

HAV is classified as a picornavirus, and most wild-type HAV does not produce a cytopathic effect, although infectious units can be quantitated by the radioimmunofocus assay (RIFA).¹¹ We describe the cell culture isolation of HAV from drinking water implicated as the source of virus transmission in a common source outbreak.

Methods

Epidemiologic Investigation

A 21-year-old man had onset of serologically confirmed hepatitis A on July 10, 1982 while living from July 9 through July 23 in a trailer park in Bartow County, Georgia. Approximately three to nine weeks following onset of illness, other residents of the trailer park developed hepatitis A. All cases of hepatitis A during 1982 were identified using county health department case reports and laboratory records for hepatitis A serologic testing. Each trailer park resident was interviewed and a blood sample obtained. All serum samples were tested by radioimmunoassay for total and IgM-specific anti-

body to HAV (HAVAB and HAVAB-M, Abbott Laboratories,* North Chicago, IL).

Case Definition

A case was defined by a positive serologic test for IgM anti-HAV. One trailer park resident who declined a blood test but reported a history of jaundice and abdominal pain was also included.

Water Sampling

Tap water from the trailers was tested for fecal coliform bacteria by standard methods.¹² Fluorescein dye was discharged from the toilet of the index case, and tap water samples were collected after 19 and 81 days.

Three months after onset of illness in the index case, 400 and 1600 liters of ground water from the well pump supplying the park were passed through an electropositive charge-modified, 25-cm long, cellulose-diatomaceous earth composite, microporous filter (Virosorb 1MDS; Cuno, Meriden, CT).

Cell Cultures

Adsorbed viruses were eluted from the filter cartridges with 1 liter of 3 percent beef extract adjusted to pH 10, and after elution, sufficient 1M glycine was added to lower the pH to 3.5. The flocculated organic material was processed according to the method of Katzenelson, *et al.*¹³ Virus culture was carried out in four monkey kidney cell lines: BGM, BS-C-1, FRhK-4 (American Type Culture Collection, Rockville, MD) and MA104 (Microbiological Associates, Bethesda, MD); and in primary Rhesus monkey kidney (PMK) cells.

The concentrated sample was filter sterilized directly onto the cell monolayer, and virus was adsorbed at 37°C for two hours with periodic rocking. PBS mock-infected cell cultures were included for controls. All cells except FRhK-4 were grown in Eagle's minimum essential medium containing Earle's balanced salts solution and supplemented with 10 percent fetal bovine serum, 25 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid), 2 mM L-glutamine, 0.1 mM nonessential amino acids, 0.2 percent sodium bicarbonate, and antibiotics (20 mcg/ml gentamycin, 100 mcg/ml streptomycin, and 100 U/ml penicillin). FRhK-4 cells were grown in William's medium E as previously described.¹⁴ Maintenance media consisted of the same basal medium

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*Use of trade names is for identification purposes only and does not imply endorsement by the Public Health Service, US Department of Health and Human Services, or the Georgia Department of Human Resources.

containing 2% fetal bovine serum. All media and media components were obtained from Gibco Laboratories (Grand Island, NY).

Continuous cell lines were passaged twice at 30-day intervals and four times at 21-day intervals. Virus was recovered from PMK cells by trypsinizing, freeze-thawing, sonication, extraction with 2,3,5 trichloro-trifluoroethane (Dupont Chemicals, Wilmington, DE), and passaged acutely.

Assays for HAV

Trypsinized cell samples were extracted as described above and assayed for HAVAg with a double-antibody sandwich enzyme immunoassay (EIA), as previously described.¹⁵ A ratio of positive to negative values (P/N) >2.1 was considered positive for HAVAg.

Infectious units of HAV were quantitated by the RIFA, using both human and rabbit anti-HAV.¹¹ HAV titers were expressed as radioimmunofocus-units (RFU) per ml.

Results

Epidemiologic Investigation

Thirty-five cases of hepatitis A with onset of illness from July 10 to November 24, 1982 were identified in the county. Before July 10, there had been no reported hepatitis A cases in the county during 1982. Of the 35 cases, 17 were residents of the trailer park. In 31 cases the date of onset of symptoms could be recalled (Figure 1). Among the 13 residents of the trailer park who could recall date of onset of illness, 12 became ill within 22 to 41 days following disease onset in the index case.

The index case was a 21-year-old male who lived with a family in the trailer park from July 9 to July 23. He denied contact with the seven other families in the park. Of the 30 trailer park residents, seven had serologic evidence of prior exposure to hepatitis A. The five persons living with the index case received immunoglobulin prophylaxis, and one became IgM anti-HAV positive. Among the 18 remaining trailer park residents considered serosusceptible to hepatitis A, 16 developed hepatitis A (89 percent attack rate). The epidemiologic investigation revealed no common social activity, meal, or food which could account for the outbreak. The only two serosusceptible individuals who did not become ill were 11 and 18 months of age, and their mothers were positive for total anti-HAV but negative for IgM anti-HAV.

Of the 18 cases who were not residents of the trailer park (including the index case), 11 had visited the trailer park and

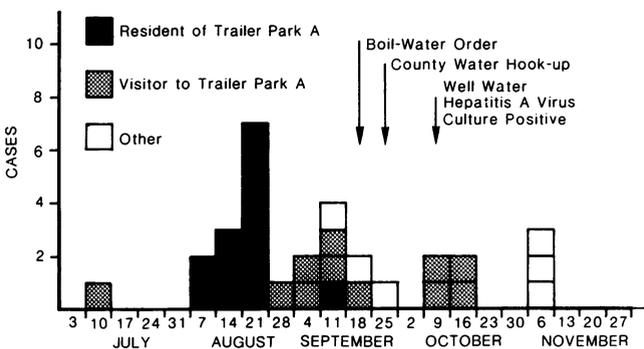


FIGURE 1—Onset of Reported Hepatitis A Cases with Known Date of Onset in Bartow County, Georgia, July through November 1982. (A county order to boil water was issued September 17. The trailer park was connected to the county drinking water supply on September 24. The hepatitis A virus-containing water samples were collected from the implicated well on October 6.)

drunk water during their visit (Figure 1). Another four patients had contact with cases only outside the trailer park, while three had no known contact with other cases.

Environmental Investigation

The trailer park's sole water supply was a 15-cm diameter drilled well having a depth of 24 to 30 m, encased within steel pipe. The trailers encircled the well at a distance of 18 to 60 m, and water was distributed through a 2-cm galvanized pipe buried approximately 50 cm below the ground surface. Wastewater from each trailer was treated by individual septic tank systems having gravity feed, subsurface drainage tile distribution networks; each septic tank system was separated from the well by a distance of 30 to 60 m.

The concentration of fecal coliforms in tap water ranged from zero to greater than 16 per 100 ml and in water collected from the wellhead was 2 per 100 ml. Fluorescein dye was flushed from the toilet of the trailer in which the index case had lived, but could not be detected on days 19 and 81 after charging the system.

A boil-water order was issued on September 17, and on September 24 the well was disconnected, and the water distribution system was connected to the county water supply.

Virus Assays

HAVAg could not be detected in the ground water concentrates by EIA. Following amplification in cell culture, HAVAg was detected in the monkey kidney cell lines (Table 1). After primary cell adaptation to FRhK-4 and PMK cells, foci of HAV were detected by RIFA (Figure 2). Passage in FRhK-4 and PMK cells yielded titers of 3.0×10^7 RFU/ml and 2.0×10^7 RFU/ml, respectively.

Discussion

The importance of HAV transmission by water is probably underestimated since outbreaks appear to be infrequently reported, poorly documented, and the virus has been only rarely isolated from this source.^{9,10,16} Using currently available methods for enterovirus detection, HAV can be concentrated 100- to 10,000-fold onto microporous filters and concentrated another 10- to 100-fold by organic flocculation.^{10,16,17} In one study in which these concentration techniques were used, samples of ground water implicated in a large outbreak of hepatitis A revealed low levels of HAVAg by RIA.¹⁶ In contrast to a previously reported outbreak where HAV was isolated from contaminated water,¹⁰ the fecal coliform counts obtained at the peak of the presumed period of contamination in the present outbreak were 10- to 100-fold lower, suggesting

TABLE 1—Detection by Enzyme Immunoassay of Hepatitis A Virus Recovered from Ground Water following Growth in Monkey Kidney Cells. (Ground water samples were collected October 6, 1982 from a well in a trailer park in Bartow County, Georgia.)

Monkey Kidney Cell Line	Hepatitis A Virus Antigen by Enzyme Immunoassay ^a by Passage Level					
	1 ^b	2	3 ^c	4	5	6
MA104	<1	<1	<1	<1	<1	<1
BGM	<1	1.1	2.4	3.6	6.9	21.0
BS-C-1	<1	2.9	8.5	11.0	21.0	>115.0
PMK	1.5	3.2	8.5	11.0	41.3	>115.0
FRhK-4	<1	2.4	5.6	10.6	57.0	160.0

^aHAVAg expressed as positive/negative (P/N) ratios in undiluted cell lysates

^bPassages 1 and 2 at 30 days

^cPassages 3-6 at 21 days

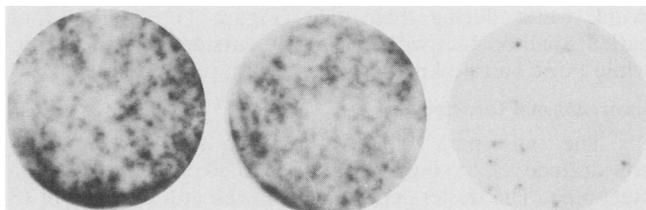


FIGURE 2—Foci Produced in BS-C-1 Cells in the RIFA from HAV Isolated from Concentrated Ground Water in Bartow County, Georgia following Initial Isolation in FRhK-4 Cells. (Panels from left to right represent increasing dilutions of the recovered HAV.)

that low levels of ground water contamination can produce significant outbreaks of hepatitis A.

This outbreak also demonstrated the stability of HAV in untreated water. The water samples from which the virus was isolated were obtained three months after the onset of symptoms in the index case and 28 days after the onset of symptoms in the last trailer park resident (Figure 1). The recovery of HAV suggests that the contaminating virus had continued access and persisted in the aquifer supplying the well although it cannot be ruled out that fourth generation cases (illness onsets from October 4 to 13) who were visitors to the trailer park, may have recontaminated the aquifer. In addition, the water concentrates were stored at 4°C for eight months before they were inoculated into cell culture.

While the incidence of HAV infection has been decreasing in the US over the past 10 years, it continues to be the second leading cause of viral hepatitis. Although the majority of the population utilizes municipal drinking water, it is evident that well water contamination with HAV continues to occur and that this source of infection is not widely appreciated.

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Review Criteria for Schools of Public Health Funding, Project Grants

Final review criteria, funding priority, and special consideration for special project grants to Schools of Public Health were published in the February 8 issue of the *Federal Register*. Among the review criteria to be considered is whether the project contains realistic objectives as described in *The Future of Public Health, Objectives for the Nation and the Sixth Report to the President and Congress on the Status of Health Personnel in the United States*.

The funding priority is designed to promote health initiatives that will encourage and increase involvement of underrepresented minority health trainees. Special consideration will be given to applications that address five issues:

- Substance Abuse
- HIV/AIDS
- Geriatrics
- Environmental health
- Training students to work in public health agencies

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