

OUTBREAK OF AMEBIASIS IN TBILISI, REPUBLIC OF GEORGIA, 1998

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Abstract: In 1998, we investigated a suspected outbreak of amebic liver abscesses caused by *Entamoeba histolytica* in the Republic of Georgia, using a case-control study. A questionnaire was administered and blood samples were obtained from cases and controls for serologic diagnosis. Medical records showed that *E. histolytica* infections were rarely diagnosed before 1998. However, from July through September 1998, 177 cases of suspected amebiasis were identified. Of 52 persons who had diagnosed liver abscesses, 37 (71%) were confirmed serologically to have antibodies against *E. histolytica*, compared with 11 of 53 persons (20.8%) diagnosed with intestinal amebiasis. In addition, 9–14% of asymptomatic controls were seropositive. Logistic regression identified the fact that interruptions in the water supply, decreases in water pressure, and increased water consumption were significantly associated with infection. The data support the hypothesis that drinking water was the source of infection, either because of inadequate municipal water treatment or contamination of municipal water in the distribution system.

INTRODUCTION

On August 19, 1998, the Minister of Health of the Republic of Georgia requested assistance from the Centers for Disease Control and Prevention (CDC) in investigating a possible outbreak of amebiasis in the city of Tbilisi. At that time, there were at least 120 cases of suspected amebiasis; more than 90 of the victims had been diagnosed with a liver abscess, and 30 had undergone surgery. Drinking water was suspected as a principal source of infection.¹ The purposes of the epidemiologic investigation were to confirm an outbreak of invasive amebiasis in Tbilisi and to identify risk factors for infection.

The Republic of Georgia is located south of Russia and is bounded on the east by Azerbaijan, on the south by Armenia and Turkey, and on the west by the Black Sea. Tbilisi is its capital. The republic declared its independence from the former Soviet Union in 1990 and experienced a civil war until 1992, when it became an independent country. The population of Tbilisi's 10 districts is estimated to be 1.5 million. The city is divided into "Northbank" and "Southbank" areas by the Mtkvari River (Figure 1).

MATERIALS AND METHODS

Case finding. Cases of intestinal and extraintestinal amebiasis were reported to the Ministry of Health by local hospitals and area physicians. Records provided by the Institute of Infectious Diseases identified the number of people undergoing ultrasound scans of the liver from January to August 1998 and the number who had lesions consistent with amebic liver abscess.

Case-control study. Case and control selection. One hundred five serum samples collected by the National Center for Disease Control in Tbilisi were sent to the Centers for Disease Control and Prevention (CDC) in Atlanta for serologic confirmation of invasive amebiasis. Cases were defined as residents of Tbilisi who had a positive serologic test for amebiasis and at least one symptom or sign (including fever, diarrhea, abdominal pain, positive scan for a hepatic abscess) consistent with invasive amebiasis (intestinal or hepatic) during the period from June 1 to September 30, 1998. Three control groups were selected for comparison: 1) neighbor-

hood controls—neighbors of cases; 2) matched controls—people randomly selected from polyclinic registers who were matched to cases by district of Tbilisi, age, and sex; and 3) Southbank controls—people living south of the Mtkvari River who also were randomly selected from polyclinic registers. Controls could be of any nationality, had to be age 20 or older (except for controls matched to cases by age), had to have lived in Tbilisi during the period from January 16 to August 1, 1998, and could not have traveled outside Tbilisi for more than 4 weeks from January 16 to August 1, 1998. In addition, controls could not have experienced an episode of diarrhea (three or more loose stools over 24 hours) lasting more than 5 days or have been told by a doctor that they had a liver abscess or amebiasis in the 6 months before the study interview. All controls were told that their participation was voluntary and that it required that they provide a blood specimen, which was collected at the time of interview.

Data collection. A standardized questionnaire was given to all cases and controls during home visits by the investigative team. Data collected included information about demographics, employment, leisure activities, food and beverage sources and preferences, water consumption, household and workplace water supply, frequency of water supply disruption to the home, and travel history. In addition, cases were interviewed about details of their illness, and a standardized form was used to abstract their hospital records for additional information about their illness. All interviews were conducted during the period from October 6 through October 21, 1998.

Data analysis. Univariate analyses were conducted to compare case responses with responses from each of the three control groups. After noting similarities in responses from the neighborhood and matched controls, we combined the two into one control group to improve statistical power in the analyses. All analyses reported here were adjusted for sex and age, unless otherwise noted. Conditional logistic regression was used to generate odds ratios (ORs) and 95% confidence intervals for potential risk factors.

Environmental assessment. Tbilisi's municipal water supply was evaluated as a possible source of infection. We visited source water supplies and water treatment facilities, and reviewed water treatment processes and records at one of the

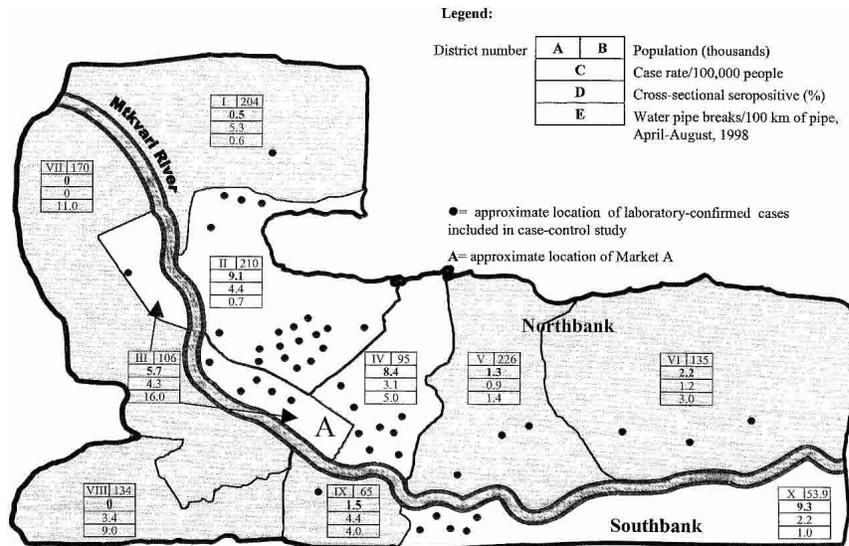


FIGURE 1. Locations of cases included in case-control study, seropositive persons in cross-sectional study, and water pipe breaks by district in Tbilisi, Georgia.

city's two surface water treatment plants, as well as city records on water quality (chlorine residuals and turbidity) in the distribution system. We also reviewed rainfall data for May, June, and July for the cities of Tbilisi, Dusheti, and Pasaunauri. We obtained data about the frequency of water and sewage pipe breaks and the locations of large water pipe (> 100 mm diameter) breaks in the city from April through August 1998. We also obtained maps depicting areas of the city where water service is routinely provided for 8, 16, or 24 hours each day.

We also visited several markets and observed how food was handled and displayed, and noted the availability of toilets and hand-washing stations.

Seroprevalence studies. *Case follow-up.* Persons enrolled in the case-control study described above gave blood samples approximately 6 months after the study. Initial serum specimens from the study were retested with the second specimens to assess changes in antibody reactivity to amebic antigens.

Cross-sectional survey. A cross-sectional study of 1,000 residents representing all 10 districts of Tbilisi was conducted 6 months after the initial outbreak. The sample size for each district was proportionate to the percentage of Tbilisi's population living in the district. Candidate participants were identified by randomly selecting addresses from polyclinic registers in each district. The sex and age category of the study participant to be interviewed at each address was randomly selected beforehand to ensure equal representation. All study participants gave informed consent, acknowledged that their participation was voluntary, and agreed to provide a blood specimen.

At the time of the household visit, a questionnaire was administered to collect demographic data and information about risk factors for amebiasis. Participants also were questioned about having been diagnosed with amebiasis or an illness consistent with amebiasis in the 6 months before the interview. Study participants also were asked to submit stool samples for parasite analysis.

Specimen collection. Blood samples were collected in tubes containing EDTA anticoagulant. After centrifugation, serum

was decanted into 2-mL vials and refrigerated for 1–6 weeks until transportation to CDC for testing. When possible, stool samples were collected from cases and other persons suspected of having amebiasis. Stool samples were preserved in formalin and/or polyvinyl alcohol (PVA) and transported to CDC for examination by microscopy.

Laboratory methods. The LMD Amebiasis ELISA kit (Allexon-Trend, Inc., Ramsey, MN) was used to test serum specimens for antibodies to *E. histolytica*. Test sensitivity is 97% for patients with extraintestinal amebiasis (liver abscess, ameboma), 77% for patients with invasive intestinal disease (ulcerative colitis, diarrhea with blood), and 71% for patients with amebic diarrhea (CDC, unpublished data). Test specificity is 95%.

Preserved stool samples were processed by concentration and permanent stain. Specimens fixed in 10% formalin were concentrated by the formalin-ethyl acetate sedimentation technique and examined by wet mount for helminths and protozoa (22-mm coverslip area).² Permanent smears of specimens fixed in PVA were stained with trichrome and examined by light microscopy for protozoa by observing 200 oil-immersion fields.²

The TechLab (Blacksburg, VA) *E. histolytica* II test (designed to detect *E. histolytica* antigens in stool specimens) was performed on stool specimens according to the manufacturer's instructions. Briefly, assay microtiter wells were incubated with 0.1 mL of diluted specimen (stool diluted 1:1 in kit diluent) and 1 drop of mAb-enzyme conjugate for 2 h at room temperature. The contents of the well strips were then shaken out and washed four times. Residual liquid was then removed by striking the strip once against a paper towel, substrate solutions were added, and the strip was incubated at room temperature for 10 min. Intensifier was then added, and after an additional 10-minute incubation, the well strips were read in a microtiter plate reader (Titertek Multiskan, Flow Laboratories, McLean, VA) at 450 nm. A positive result was defined as an optical density reading > 0.05 after subtraction of the negative control optical density.

RESULTS

Case finding. From 1972 through 1997, *E. histolytica* infections were rarely diagnosed at medical facilities in Tbilisi. Anecdotal reports indicated that medical centers throughout Tbilisi treated only 1 or 2 patients a year with amebic liver abscess. By the end of August 1999, the Ministry of Health had received reports of at least 120 cases of suspected amebiasis, of which more than 90 patients had been diagnosed with amebic liver abscess. Data provided by the Institute of Infectious Diseases in Tbilisi indicated that from January 1 to July 31, 1998, none of the 52 persons undergoing ultrasound scans of the liver were positive for lesions consistent with amebic liver abscesses, compared with 43 of 164 persons (26.2%) scanned from August 1 to September 17, 1998.

Cases included in the case-control study were from throughout the city, with the highest case rates occurring in districts X, II, IV, and III (Figure 1). Only two districts (VII and VIII) had no cases.

Case-control study. Participant characteristics. Of the original 105 serum samples sent to CDC in Atlanta for diagnosis, 52 were from persons diagnosed with amebic liver abscesses and 53 from persons with intestinal amebiasis. Of the 52 reported cases of liver abscesses tested at CDC, 37 (71.2%) were seropositive, compared with 11 (20.8%) of 53 reported cases of intestinal infections.

Forty-six of the 48 persons with a positive serologic test were included as cases in the case-control study. Two seropositive persons were excluded from the case-control study because they could not be located. Forty-eight neighborhood controls of cases were interviewed, as were 47 matched controls and 50 Southbank controls. Blood samples were obtained from all persons in each control group.

Of the 46 cases included in the study, 35 (76.1%) were male and 11 (23.9%) were female; 36 (78.3%) were diagnosed with liver amebic abscess and 10 (21.7%) with intestinal amebiasis. Six patients included in the study had undergone surgery, five with liver abscess and one with intestinal disease. All the patients were citizens of Georgia, and most (89.1%) were of Georgian ethnic origin. Their ages ranged from 6 to 74, with a median of 49 (Table 1). Neighborhood controls and Southbank controls had a similar age range and median. The age and sex distribution of matched controls was similar to that of the cases. Neighborhood controls were distributed evenly be-

tween males and females. There was an unexplained high number of females among the Southbank controls.

Illness onset dates reported by the cases are shown in Figure 2. All patients were hospitalized. After onset of illness, 16 of the abscess patients (44.4%) and five patients with intestinal disease (50%) reported that they had attempted self-medication before seeing a physician. The most common medications were non-steroidal anti-inflammatories and antibiotics such as gentamicin, ampicillin, and chloramphenicol, all available without a prescription. No patients initially took anti-protozoals such as metronidazole or tinidazole.

Among cases diagnosed with hepatic amebiasis, 15 (41.7%) reported diarrhea, and 35 (97.2%) reported fever. Among diagnosed cases of intestinal amebiasis, all 10 reported diarrhea; 8 (80%) had bloody diarrhea. Two persons with intestinal disease (20%) reported fever.

Stool analysis. A total of 54 stool samples were collected. Three samples were from patients in the case-control study who had been treated for amebiasis; the rest were from suspected cases receiving care in local hospitals. Twenty-eight samples were preserved in both formalin and PVA, 16 in formalin only, and 10 in PVA only. Attempts to isolate the organism from fresh, unpreserved stool samples were unsuccessful, though one case was confirmed to be *E. histolytica* by antigen detection. *E. histolytica/dispar* trophozoites and cysts were identified in four of the 54 stool samples (7%). Of the three samples from cases, one was positive for *E. histolytica/dispar*, and hepatic amebiasis had been diagnosed. No parasites were observed in the stool samples collected from the other two cases. One case had been diagnosed with intestinal amebiasis and the other with hepatic amebiasis.

Blood specimens were analyzed for 22 of the 54 persons who submitted a stool specimen. Only one of the two stool-positive persons was seropositive. Three of the seropositive persons (75%) were negative by stool exam. Fourteen samples (26%) had evidence of other parasites (*Giardia lamblia*, *Blastocystis hominis*, *Entamoeba coli*); 11 samples (20%) contained white blood cells (WBCs); two samples had red blood cells (RBCs); and four had insufficient quantities for complete analysis. Altogether, 33% of the samples contained at least one parasite, and 50% of the samples contained either a parasite, WBCs, or RBCs (suggesting infection). Ten percent of all negative stool specimens were double-checked, and

TABLE 1
Age (years) and sex distribution among cases, controls and seropositive controls, Tbilisi, 1998

Study	Age group							Median	Sex	
	<10	10-19	20-29	30-39	40-49	50-59	≥60		Male	Female
Case-control study										
Cases	1	0	6	4	13	8	14	49	35	11
Controls										
Neighbor ^a	0	0	11	9	11	5	6	41	20	21
Matched ^b	1	1	7	5	11	6	12	47	35	9
Southbank ^c	0	1	9	10	6	6	14	45	15	31
Controls combined ^d	1	1	18	14	22	11	18	44	55	30
Seropositive controls	0	0	2	1	4	3	4	49	7	7
Cross-sectional study										
All persons	8	71	192	224	162	86	131	37	312	552
Seropositive	0	0	1	2	9	8	6	48	4	22

^a Neighborhood controls
^b Controls matched by district, age, and sex
^c Controls randomly selected from the Southbank
^d Neighbor and matched controls combined

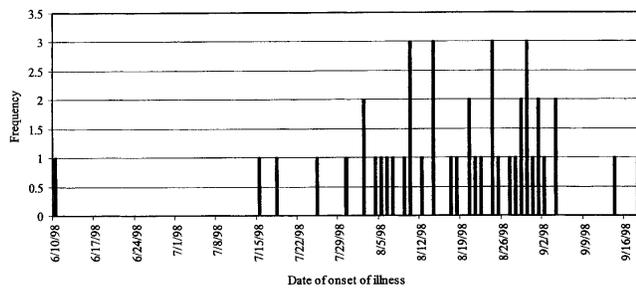


FIGURE 2. Cases of invasive amebiasis by onset of illness, Tbilisi, 1998 ($N = 44$).

all positive specimens were confirmed by a second laboratorian.

Serologic results for persons serving as controls. Fourteen of 137 people serving as controls had positive serologic tests for *E. histolytica*: six neighborhood controls (12%), four Southbank controls (9%), and four matched controls (13%). The seropositive controls had ages ranging from 22 to 75, and were evenly divided by sex (Table 1).

Data analysis. Significant risk factors were similar when cases were compared with persons in each of the three control groups. Data from the neighborhood and matched controls were combined and the statistically significant variables summarized in Table 2. All results are adjusted for age and sex unless otherwise noted.

Living conditions. Information about the number of rooms (excluding kitchen and bathroom) and number of people living in a house or apartment were obtained from cases and controls when they were interviewed. Cases were more likely to live in one-room households (26.1%) compared with neighborhood controls (14.3%) and matched controls (4.7%). A crowding index was calculated by dividing the number of people per household by the number of rooms in the household. The chances of a household's having a person with amebiasis increased 1.59 times as the ratio of people per room in the household increased by one person per room. Three cases reported that a member of the family had been diagnosed with amebiasis; however, this variable was not statistically significant in our analysis.

Home water supply. Persons who experienced interruptions in water service to their home during the period from January

15 to August 1, 1998 were 4.52 times more likely to have amebiasis ($P = 0.018$) (Table 2). While interrupted water service was slightly significant, the frequency of interruptions was not statistically different for case patients and controls.

Amebiasis also was associated with decreases in home water pressure without an interruption in flow ($OR = 1.49$; $P = 0.02$) (Table 2). Twenty-eight percent of cases reported daily decreases in water pressure compared with 7% of neighborhood controls and 5% of matched controls. Change in water in terms of taste, color, turbidity, or odor was not significant in this analysis ($P = 0.25$), and having running water at the workplace was not significantly associated with disease ($P = 0.30$).

Drinking water. Water consumption patterns for case and control study participants were significantly different. Water consumption by cases and controls was similar for February and March of 1998, but cases' consumption began to increase in April (Table 3). In June and July, differences in the amount of water consumed per day were statistically different for cases and controls. Cases were 4.63 times more likely than controls to consume larger amounts of drinking water per day in June ($P = 0.002$) and 5.96 times more likely in July ($P = 0.001$). Consumption of 7–10 glasses of water per day in June and July was reported by 52–54% of cases compared with 16% of neighborhood controls and 14% of matched controls. Seventy-six percent of cases who consumed 7–10 glasses of water per day in June or July had onset of illness in August. Thus, the large volumes of water consumed by cases were not a consequence of amebic disease (i.e., the result of fever or diarrhea) but rather a risk factor for becoming ill.

Markets and food. Analysis of questionnaire data about where participants shopped and what types of food they bought indicated that cases were 4.91 times more likely than controls to visit one particular government-regulated, or official, market (Market A) (Table 2). Thirty-five cases (76.1%) shopped there compared with 27 neighborhood controls (64.3%) and 26 matched controls (60.4%). Purchasing dairy products at any official market was also a significant risk factor for amebiasis ($OR = 3.74$), as was purchasing dairy products from a street vendor ($OR = 2.99$). In an analytic model that excluded purchasers of dairy products from a street vendor, persons who bought dairy products at an official market were 9.91 times more likely to have amebiasis ($P = 0.0015$) than those who purchased dairy products from other sources.

TABLE 2

Odds ratios (OR), P values, and 95% confidence intervals for those variables significant in analysis for combined control groups and adjusted for age and sex, Tbilisi, Georgia, 1998

Variable	OR (% cases)	P value	LCL ^a	UCL ^b
Crowding index	1.59	0.034	1.04	2.43
Interruption of home water supply ^c	4.52 (84.8)	0.018	1.03	10.94
Daily decreases in pressure of home water supply	1.49 (28.0)	0.023	1.05	2.08
Purchasing vegetables at an unofficial market ^c	2.88 (15.9)	0.0005	2.88	infinite
Purchasing fruit at an unofficial market ^c	3.41 (18.2)	0.0003	3.41	infinite
Purchasing dairy products at an official market	3.74 (28.3)	0.014	1.30	10.78
Shopping at official Market A	4.91 (79.5)	0.029	1.18	20.51
Alcohol consumption	1.46 (73.9)	0.047	1.00	2.12
Consuming alcohol outside the home	1.48 (18.2)	0.045	1.01	2.19
Drinking draft beer	4.00 (65.2)	0.016	1.30	12.5

^a Lower confidence limit.

^b Upper confidence limit.

^c Based on analysis with matched controls only and not adjusted for age or sex.

TABLE 3

Percentage of cases and controls consuming seven or more glasses of water per day from February through July 1998, Tbilisi

Month of water consumption	Cases n (%) [*]	Controls n (%) [*]	OR ² /P value
February	4/46 (8.7%)	9/86 (10.5%)	0.84/.792
March	3/45 (6.7%)	6/83 (7.2%)	0.94/.934
April	7/45 (15.6%)	7/83 (8.4%)	1.80/.350
May	13/45 (28.9%)	11/83 (13.2%)	2.20/.129
June	24/45 (53.3%)	14/83 (16.9%)	4.63/.002
July	25/45 (55.6%)	14/85 (16.5%)	5.96/.001

^{*} Number and percentage drinking ≥ 7 glasses of water per day compared with those drinking 0-6 glasses per day.

² Odds ratio: Calculated by conditional logistic regression and adjusted for age and sex.

Likewise, when purchasing dairy products was analyzed independently of purchasing dairy products at an official market, the OR was 7.87 ($P = 0.0026$). Dairy products included milk, cheese, and yogurt. Although we did not include follow-up questions in the questionnaire to help identify particular dairy products that may have been responsible for this association, purchasing cheese at the official market visited most frequently was not statistically significant, nor was eating cheese. Tasting cheese at markets was protective for developing amebiasis (OR = 0.25), but when the data were adjusted for shopping at official markets, the protective benefit associated with tasting cheese was not statistically significant.

Several other food items were significantly associated with amebiasis. Purchasing vegetables at an official market was not significant, but purchasing vegetables at an unofficial market was significant with an OR of 2.88 ($P = 0.0005$) (Table 2). Only seven neighborhood control cases (15.2%) purchased vegetables at an unofficial market compared with one neighborhood control (2.38%) who bought vegetables at an unofficial market and one matched control (4.65%) who purchased vegetables at an unofficial market (Table 2). Herbs were not significantly associated with disease ($P = 0.155$) (Table 2).

Buying fruit at an unofficial market was also significant, with an OR > 3.41 ($P = 0.0003$) (Table 2). Eight cases (17.4%) purchased fruit at an unofficial market compared with one neighborhood control (2.38%) and one matched control (4.65%). Unfortunately, we were not able to ask detailed questions about all types of fruit purchased, but we did

ask about a few specific fruits such as apricots, peaches, strawberries, raspberries, and cherries. Eating these fruits was associated with a decreased risk of developing amebiasis, with P values ranging from 0.01 (cherries) to 0.0023 (apricots).

Alcohol. Monthly or more frequent consumption of alcohol was significantly associated with developing amebiasis (OR = 1.46; $P = 0.047$) (Table 2). Ten cases (21.7%) consumed alcohol once a year or less compared with 15 neighborhood controls (35.7%) and 12 matched controls (27.9%). Thirty-four cases (73.9%) consumed alcohol once a month or more compared with 27 neighborhood controls (64.3%) and 31 matched controls (72.1%). Frequency of consuming alcohol outside the home was also significantly associated with disease (OR = 1.48; $P = 0.045$). Although consuming bottled beer was not significant in the analysis, drinking draft beer was (OR = 4.00; $P = 0.016$). Drinking draft beer at a local brewery (Brewery A) was borderline significant in the analysis (OR = 2.33; $P = 0.092$). Twenty-two cases (47.8%) reported drinking draft beer from Brewery A, compared with 14 neighborhood controls (33.3%) and 14 matched controls (32.6%). Drinking draft beer from another local brewery (Brewery B) was not significant in the analysis. Consuming alcohol remained significant when only hepatic amebic cases were included in the analysis.

Additional variables. No association was observed for several other variables including eating fruits, vegetables, or herbs grown in a garden; washing fruits and vegetables; eating in a restaurant or eating food sold by a street vendor; visiting public baths or saunas; and fishing. Certain behaviors at the market were also not significant, including sampling vodka, fruit, vegetables, or fish and using a public restroom.

Environmental assessment. Tbilisi obtains its water from three sources: wells, groundwater derived from sand infiltration galleries along the Aragvi River, and surface water that originates from Zhinvali Reservoir northwest of the city (Figure 3). Groundwater sources supply about 80% and surface water about 20% of Tbilisi's water. All sand infiltration groundwater supplies are chlorinated. The well water supply was not chlorinated before the outbreak. Surface water is detained in a small storage reservoir (Bordona) several miles downstream from the dam. From there, it is piped through a mountain to two treatment plants near Tbilisi where it undergoes sedimentation, rapid sand filtration, and chlorination.

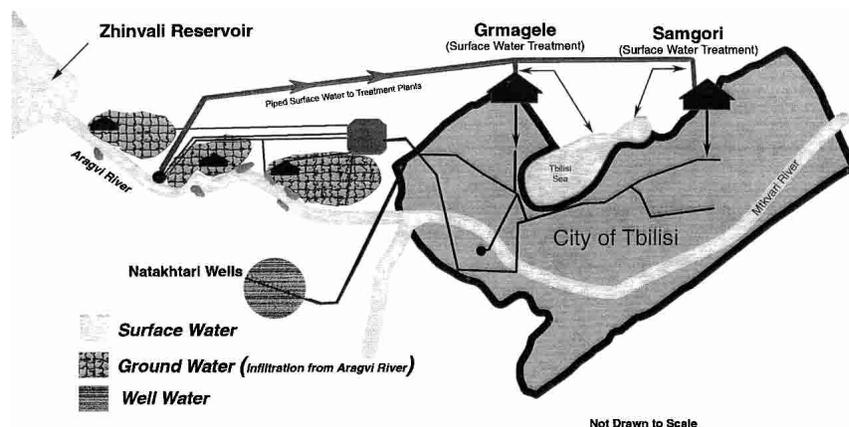


FIGURE 3. Diagram showing Tbilisi's three water sources and two water treatment facilities, 1998.

Excess water arriving at the treatment plant overflows into an open holding reservoir (Tbilisi Sea), which serves as an emergency water source during droughts.

Treated water from the two surface water treatment plants mingles in various parts of Tbilisi's water distribution system with well water and groundwater from the Aragvi River infiltration galleries. The proportion of well, ground, and surface water in the mixture supplied to each area of the city was not known, and test equipment was not available in Tbilisi to measure it. The prevailing opinion of Tbilisi municipal employees was that surface water predominated in most areas north of the Mtkvari River ("Northbank") and groundwater predominated south of the river. However, water pipes cross the river at several locations, allowing some distribution of groundwater north of the river and some surface water south of the river.

Large pipes (> 100 mm in diameter) in the water distribution system and sewage system required over 200 repairs per month from April 1 through August 31, 1998; this was most common in districts III (16 breaks per 100 km of pipe), VII (11 breaks/100 km), and VIII (9 breaks/100 km) as shown in Figure 1. Twenty-six areas of the city are provided with running water for only 8–16 hours a day.

Several small villages and a resort community are located in the drainage basin for Tbilisi's main reservoir (Zhinvali). These communities either discharge untreated sewage directly into streams that empty into the reservoir or have sewage disposal systems that let sewage be washed into the reservoir during heavy rains. In May, storms produced 23 mm of rainfall on May 12 and another 16 mm six days later at the upper end of the reservoir (Pasanauri). Three weeks later, another 31 mm of rain fell in the same area during a three-day period. During May and June, similar amounts of rain fell near the Zhinvali dam and at a surface water holding pond just below the dam at Bordona. Records show that fecal coliform levels at the dam were consistently > 1,100 coliforms/L in June and July. Coliform testing of reservoir water did not include counting the number of coliforms greater than the 1,100 coliforms/L standard. Thus, it was not possible to determine when the highest levels occurred or to assess whether there was a single fecal contamination event or multiple ones.

Review of the records from one of the two surface water treatment plants (Grmagele) revealed that the water averaged around 16°C and had a pH of 7.5, and that fully treated water leaving the plant routinely had a chlorine residual of 0.5 mg/L and turbidity of 4.0–5.0 NTU before August 25. Calculation of the probable microbial exposure time to chlorine disinfection was based on the free chlorine residual in water at the water treatment plant (0.5 mg/L), length of time that treated water is held at the plant (30 minutes), and the time it takes water leaving the treatment plant to reach the first household taps (97 minutes). The product of free chlorine residual (mg/L) multiplied by the number of minutes that an organism is exposed to chlorine before water is consumed (commonly called the disinfection contact time or CT) was calculated to be 63.5 mg&z.ccirf; minutes/liter. If drinking water were contaminated within the distribution system, the parasite would be subject to an even lower disinfection CT and would have an even greater chance of surviving until it reached a household tap. The minimum CT needed to kill *E. histolytica* under Tbilisi water conditions (pH and tempera-

ture) was calculated to be 76.4 mg&z.ccirf; minutes/liter for chlorine-demand free water.^{3,4}

Three major official markets were visited, including Market A. Some markets had both indoor and outdoor vending booths. A range of unsatisfactory sanitary conditions was observed, including fewer hand-washing stations than recommended by the Ministry of Health for markets, absence of soap at many hand-washing stations, and no running water in the toilets at Market A. Fruit and produce were usually displayed on open table tops without covering. These items were kept fresh by either being stored in buckets of water or periodically sprinkled with water. Water used to freshen produce was often stored in an open container, and bare hands were used to splash the water on the produce. A few vendors fashioned water sprinklers out of plastic bottles that had been perforated with a number of small holes; when such devices were used, there was less opportunity for water contamination.

Seroprevalence studies. Cases. Twenty of 46 cases included in the case-control study were re-examined for persistence of anti-*E. histolytica* antibodies 6–8 months after the first serum sample was obtained. In all cases, the first sample was collected when the patients first sought medical care for their illness. Twenty-six of them were not included in the second blood drawing either because they either could not be located or did not agree to participate. Of the 20 with paired sera, all were serologically positive at the time of their illness (optical density [OD] ≥ 0.500). At the time of the second blood draw, 11 (55%) were seronegative (OD < 0.500). The percentage of persons seroconverting to negative was similar for those with hepatic infection (9/26 [53%]) and those with intestinal infection (2/3 [66%]).

Cross-sectional study. A total of 879 people were enrolled in the cross-sectional study during February and March 1999. All were residents of Tbilisi. Figure 1 shows the distribution of study participants by district of Tbilisi compared with the distribution of the general population throughout Tbilisi. Of the 879 study participants, 552 were women (63%), 312 were males (35%), and the sex of 15 (2%) was not recorded. Ages ranged from 8–89 years, with a median of 37 (Table 1). Although only eight (0.9%) reported having been diagnosed with amebiasis (intestinal or liver) in the past, 101 (11%) had experienced diarrhea (three or more loose stools in 24 hours) since January 1, 1998.

Of the 879 study participants, 26 (3.0%) had antibodies to *E. histolytica* with an OD reading of ≥ 0.5 . Seroprevalence rates on the Northbank and Southbank of the city were 3.2% and 2.3%, respectively. The distribution of seropositive persons by district was similar to the distribution observed for cases enrolled in the case-control study. Persons living in districts II, III, IV, and X (Figure 1) were 1.52 times more likely to be seropositive than those living in other districts of Tbilisi (3.7% versus 2.4%). However, this difference was not statistically significant. One hundred seventy-seven participants submitted stool samples for microscopic analysis and stool antigen testing. By microscopic analysis, four specimens contained *E. histolytica/dispar* but 51 (28.8%) contained at least one parasite (*E. histolytica/dispar*, *B. hominis*, *E. coli*, *Endolimax nana*, *Entamoeba hartmani*, and *Iodamoeba butschlii*). Three stool samples were positive by stool antigen testing. Two of these also had *E. histolytica/dispar* identified by microscopy, and one was serologically positive.

DISCUSSION

A large outbreak of amebiasis occurred in Tbilisi during the summer of 1998. Anecdotal reports and ultrasound records for the year suggest that amebiasis was rarely diagnosed in Tbilisi before June 1998, but reports of liver abscess consistent with a diagnosis of amebiasis increased during the summer months. The high seropositive rate (10%) among controls in the case-control study conducted in September and October 1998 suggests that the outbreak was widespread and may have affected 84,000–225,000 people. The finding of a 3% seroprevalence rate during the cross-sectional study conducted 6 months later suggests that the outbreak had begun to wane. Although rapid decline in antibodies after *E. histolytica* infection is not typical,^{5,6} this has been observed in Bangladesh by one of the authors (WAP, unpublished data). The documented change from seropositive to seronegative in > 50% of cases within 6–8 months of diagnosis supports the hypothesis that a rapid decrease in seroprevalence could occur in the absence of a high rate of continued transmission. Thus, the observed differences in seroprevalence rates (10% and 3%) in the two samples of Tbilisi's population obtained by different methods may reflect a real change in community seroprevalence during that short period of time. Alternatively, the larger sample size of the population surveyed in the cross-sectional study may be a more-accurate overall estimate of seroprevalence.

Unusual aspects of this outbreak of amebiasis were the detection of a high number of persons with liver abscess and the small number of children who met the case definition. Liver abscesses normally occur in about 10% of people infected with *E. histolytica* and are more common in men aged 50 or more.^{5,7} In this outbreak, most cases reported to the Ministry of Health (80%) had serologically confirmed liver abscess. We believe this is why most of our patients were adults. The demographics of the population with liver abscess is consistent with those reported in the literature, i.e., most are men (80%) and the median age is 49 (Table 1). The high number of cases of liver abscess detected may be a consequence of either a failure to diagnose many of those with intestinal illness or reluctance of persons with intestinal illness to seek medical attention. Microscopy was used in Tbilisi to diagnose intestinal cases; such methods can have low sensitivity and do not distinguish between *E. histolytica* and non-pathogenic *E. dispar*.⁵ In addition, special stains and ocular micrometers were unavailable in Tbilisi, making it even more difficult for laboratory technicians to detect or distinguish *E. histolytica* from other *Entamoeba* species or artifacts. It was also common for persons with this infection to self-medicate, which can lead to delayed diagnosis, misdiagnosis, and under-reporting. The rapid decline in serum antibody postinfection and frequent occurrence of liver abscess also raised questions about whether an unusual strain of *E. histolytica* could have caused this outbreak. It would be interesting to further characterize the *E. histolytica* isolate(s) responsible for the outbreak, as there are no molecular markers for strains prone to cause invasive disease.

Cases were widely found throughout Tbilisi (Figure 1). Thirty-four patients lived on the Northbank and 12 on the Southbank. The highest case rate per 100,000 people occurred in four contiguous districts, X, II, IV, and III, with case rates of 9.3, 9.1, 8.4, and 5.7, respectively. Eighty-three percent of

all cases came from these four districts. The old city of Tbilisi overlaps districts VIII and IX and part of VII, which had very low case rates. We were unable to determine if there was a bias in detection of cases in these districts compared with other districts; however, the seropositive persons from both the case-control study and the cross-sectional study came from similar geographic areas (Figure 1), suggesting that the risk of infection was higher for people living in these areas of Tbilisi.

The clinical features of those with amebiasis were consistent with those reported in the literature.^{5,7} Diarrhea was reported in all intestinal cases and 42% of liver abscess cases. Fever was reported in 97% of the abscess cases but only 20% of the intestinal cases. Although 81% of the abscess cases reported change in skin color, which is higher than the rate observed in most patients with amebic liver abscesses, we could not determine if the change was caused by jaundice or paleness associated with illness.

Recognized modes of *E. histolytica* transmission include person-to-person, food, and water. In this investigation, we found evidence of all three. However, available data indicate that contaminated drinking water was the most likely cause of the outbreak, either through inadequate municipal water treatment or contamination of water in the distribution system. First, there were opportunities for fecal contamination of the reservoir that supplied surface water to Tbilisi. Several small towns and villages have been built on the mountain slopes that surround the reservoir. Sewage runoff and storm sewer overflow from these communities drain downward toward the reservoir. Heavy rains and higher-than-acceptable coliform counts in the months before the outbreak confirmed the occurrence of such events. Second, records collected at the surface water treatment facilities suggested that the level of filtration and disinfection provided were not adequate to remove or kill *E. histolytica* cysts (12–16 micrometers) if they were present.^{3,4} The filters at this water treatment plant produced treated water with a turbidity of 4.0–5.0 NTU before August 25. This level of filtration would allow a large portion of particles 16 μm in diameter or smaller to pass into the distribution system's water.

Third, the high frequency of water pipe breaks and scheduled interruptions in water to different sections of the city created major pressure drops that could lead to back-siphonage of sewage into the water system. Interruption of home water supply and daily decreases in home water pressure were identified as risk factors in the case-control study. In 1994, the United States Agency for International Development (USAID), estimated that the city was losing 40–60% of its water per day because of broken pipes.⁸ Although Tbilisi has scheduled interruptions in water flow to several areas of the city, most of the 46 confirmed cases (65%) lived in areas of 24-hour-per-day service. This suggests that many of the interruptions in the water supply reported by cases were caused by damaged pipes in the distribution system. The district with the highest rate of water pipe breaks (III) also had a high case rate. However, districts VII, VIII, and IX also had a large number of pipe breaks but had low case rates. These districts may have received a higher proportion of groundwater, which is at low risk for *E. histolytica* contamination, than districts closer to the surface water treatment plants. Sewage cross-connections during pipe breaks in districts VII, VIII, and IX could only contribute to further

spread of the parasite through drinking water if there were already large numbers of *E. histolytica* cysts in the sewage. Sewage levels of cysts would be expected to be lowest in those districts where few cases were detected. But sewage could not be tested for *E. histolytica* because of lack of appropriate equipment, supplies, and other resources.

Fourth, data on water consumption patterns indicated that cases were more likely to consume larger amounts of water in the 4–12 weeks before illness. Lastly, 87% of cases reported one or more statistically significant water-related risk factors, i.e., interruptions in home water supply, daily decreases in home water pressure, or drinking more than six glasses of water per day before onset of illness.

We were unable to test source water or finished water for *E. histolytica*. There are no standardized methods for examining water for *E. histolytica*, and we lacked equipment, supplies, and laboratory facilities to conduct water sampling experiments. Moreover, our investigation occurred too late in the outbreak to adequately detect the parasite without immunodiagnostic reagents and methods similar to those used to detect *Cryptosporidium* or *Giardia* in water.

In our analyses, three types of foods also were significantly associated with illness: fruit and vegetables purchased at an unofficial market, and dairy products purchased at an official market. Because these variables were highly correlated with each other, we were unable to conduct a multivariate analysis to determine which, if any, of them were independently associated with illness, i.e., in the absence of a water exposure. Thus, any of these food products could have been an independent risk factor for some of the cases. A high percentage of cases (61%) had at least one food-related risk factor. However, 89% of cases with a food-related risk factor also had a water-related risk factor, and 79% had a market-related risk factor. All but one of the cases with a food-related risk factor could be explained by a water- or market-related risk factor. Conversely, only 18% of the water-associated cases could be explained by produce and 48% by dairy products. It is not possible from this study to determine if some cases with food-related risk factors were really infected by water or if some food products implicated as risk factors were tainted by contaminated water. But although we could not determine how the implicated food products may have been contaminated, we concluded that if they were contaminated, it probably occurred locally, since the products were widely distributed in the Republic of Georgia and no outbreaks of amebiasis were reported in other cities. Two of our three leading hypotheses for how produce may have become contaminated included a water source: 1) food products may have been washed with contaminated water before they were brought to a market; 2) food products may have been washed or sprinkled with contaminated water at a market; and 3) produce or dairy products may have been contaminated directly by soiled hands or unsanitary surfaces at the market as supported by evidence of inadequate hand-washing stations and dysfunctional toilets, especially at Market A.

Several fruits were identified as protective in the case-control study. We do not believe that these fruits actually provided protection from amebiasis; more likely, eating them indicated socioeconomic status or behaviors that decreased the risk of amebiasis. Crowding was also significantly associated with illness and may also indicate socioeconomic status or secondary transmission.

Shopping at Market A was also significant in our analysis and could be explained by either poor sanitary conditions there or water contamination. Many food products sold at Market A could have been contaminated by water, soiled hands, or soiled food vending surfaces. Market A was located in district III, which is one of the four districts with the highest concentration of cases. It is adjacent to districts II and IV, two of the three other districts with the highest case rates. We could not determine whether Market A contributed to the outbreak directly or if it is significant in our analysis because it is located near districts with the highest concentrations of cases. District III had the highest rate of water pipe breaks per 100 km of pipe from April–August, 1998, and this may have contributed to contamination of water used at Market A or tap water used by residents of District III.

In conclusion, this outbreak is among the largest waterborne outbreaks of amebiasis reported.^{7,9,10} Large waterborne outbreaks of the disease have been uncommon in the past 40 years.^{7,9,10} The highest proportion of cases could be accounted for by risk factors associated with drinking water, and we believe the epidemiologic data most strongly support waterborne transmission. Although there is evidence for foodborne transmission, we do not believe food was the principal mode of transmission because many different types of food were implicated, and outbreaks of amebiasis were not detected in other communities receiving these same food items. Many of the foodborne infections could have resulted from produce contaminated by tainted Tbilisi water, inadequately washed hands, or soiled environmental surfaces at Market A. In an outbreak of this magnitude, some secondary person-to-person transmission would be expected through crowded living conditions and/or indirectly through foods or beverages handled by infected persons at home or work. This outbreak serves as a reminder that cities with decaying water treatment facilities and crumbling water distribution systems are at risk for waterborne disease outbreaks, especially with organisms that are moderately to highly resistant to chlorine disinfection. Diagnosis and epidemiologic investigation of this outbreak were encumbered by the unavailability of diagnostic test equipment and reagents in the Republic of Georgia. The obstacles encountered in investigating the outbreak further highlight the need for rapid, reliable, and low-cost diagnostic tests to detect *E. histolytica* in feces, water, and food. Isolation of the Tbilisi strain of *E. histolytica* will be needed to compare its molecular differences and similarities with other isolates, to better assess its propensity to cause extraintestinal disease, and to study host antibody responses to it.

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