



# A Case-Control Study of Timely Control and Investigation of an *Entamoeba histolytica* Outbreak by Primary Health Care in Idahluy-e Bozorg Village, Iran

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## Abstract

**Background and aims:** Experience transfer and the knowledge utilization of rapid and timely outbreak control can be the key path-finding to health systems (HSs). The aim of this study was to investigate an *Entamoeba histolytica* diarrhea outbreak through timely control.

**Methods:** A case-control study and an environmental survey were conducted to identify the source of infection and the outbreak etiological agent. Sixty-two confirmed cases in Idahluy-e Bozorg village, Iran, and 62 group-matched controls (in terms of age and sex) were selected from the healthy neighbors of the same village during the same period. Then, multiple logistic regression was applied to calculate the adjusted odds ratio for diarrhea risk.

**Results:** Totally, 250 cases were identified by a continuous common source epidemic from January 19th to 31st, 2018. Out of the total 36 stool samples, *E. histolytica* was detected as the common organism. In addition, the final analysis indicated that network drinking water (NDW) increased the odds of diarrhea (16.56, 95% CI=4.14-64.87), whereas well water and boiling water had protective effects and decreased the odds of diarrhea by 81% and 66%, respectively.

**Conclusion:** Overall, the source of infection was detected due to the contamination of underground NDW by pipe sewage erosion. Therefore, additional monitoring of water systems is necessary, especially during the summer and regarding full readiness for outbreak control.

**Keywords:** Outbreak control, *Entamoeba histolytica*, Case-control study, Health system

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## Introduction

Food and water-borne diseases (WBD) are linked to significant disease burden worldwide. About 1.7 to 5 billion diarrhea cases occur per year<sup>1,2</sup> and diarrhea causes 3.4 million deaths, the majority of which occur in children under five years old.<sup>3</sup> In developing countries, WBD is on the rise and children from these countries suffer from diarrhea three times a year, on average.<sup>4</sup> Climate change, increased international traveling, fast food consumption, tourism development, population mobility, and global food trade can increase WBD occurrence.

Amebiasis is an intestinal infection caused by *Entamoeba histolytica*. Globally, *E. histolytica* is estimated to infect about 50 million people and each year, more than 55 000 people die as a result of amebiasis.<sup>5,6</sup> In addition, this

infection is still a health problem in developing countries with low human development index and poor sanitary conditions. Among the Iranian population, the pooled prevalence of *E. histolytica* infection is about 1% (95% CI 0.8-2.0%), but a higher prevalence is observed in undeveloped districts.<sup>7</sup> Further, *E. histolytica* can cause an epidemic in insanitary conditions or the contamination of network drinking water (NDW).

System readiness, timely response to outbreaks, and their immediate control are major priorities for health systems (HSs). These parameters are also highly vital for mortality prevention and care quality.<sup>8,9</sup> Currently, HSs have produced the WBD surveillance system in the world. This system was established in Iran in 2007. The Iranian Center for Communicable Disease Control monitors the

diarrheal diseases continuously. In 2012, more than 1037 food and WBD outbreaks were reported in Iran<sup>4</sup> although the outbreak report cannot reflect the poor health status or HS failure. An immediate report of the outbreaks indicates the system sensitivity, readiness, and timely response to them. Experience transfer and the knowledge utilization of rapid and timely outbreak control are regarded as the key path-finding to HSs. Therefore, the current study sought to investigate an *E. histolytica* diarrhea outbreak through timely control by primary health care in Idahluy-e Bozorg village, Iran.

## Materials and Methods

### Study Design

A diarrhea outbreak was reported by the community health worker (CHW, health home) from Idahluy-e Bozorg village, Malekan county, Iran. Idahluy-e Bozorg is a village in Gavdul-e Sharqi rural district, in the central district of Malekan county, East Azerbaijan province, Iran (Figure 1). At the 2017 census, its population encompassed 547 people. A team of health experts including a family physician, an epidemiologist, a disease expert, an environmental health expert, a clinician, and the CHW was immediately deployed in the outbreak area by the Department of Disease Control and Prevention, Malekan Health Network on June 19, 2018 (on the reported day).

Ten steps of outbreak investigation and control were applied by our outbreak team according to the center for disease control guidelines. These steps were as follows.

1. Identifying the investigation team and resources;
2. Establishing the existence of an outbreak;
3. Verifying the diagnosis;
4. Constructing case definition;
5. Finding cases systematically and developing the line listing;
6. Performing descriptive epidemiology/develop hypotheses;
7. Evaluating hypotheses/perform additional studies as necessary;
8. Implementing control measures;

9. Communicating the findings;

10. Maintaining surveillance.<sup>8</sup>

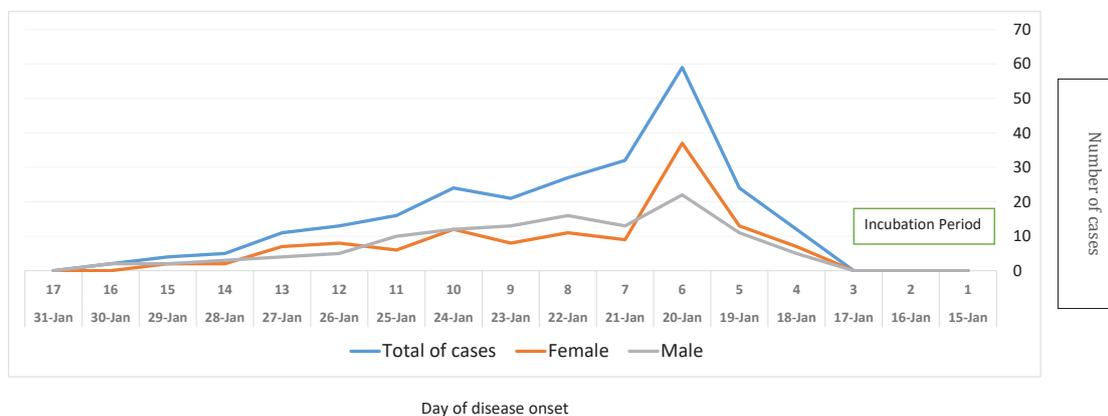
Each step of the outbreak control has a lot of details which are described in the guidelines. However, in this article, it was attempted to technically and practically explain and describe the method of launching a case-control study and how to identify the source of the outbreak.

### Outbreak Investigation

A 1:1 case-control study was conducted to identify the source of infection, the etiological agent of the outbreak, the mode of transmission, the patterns of the outbreak, and the risk factors of the infection. After obtaining ethical approval and informed consent, newly affected cases in addition to existing patients entered into the study. Finally, 62 cases were confirmed according to their hospital records or clinical signs and 62 controls were selected as well. Cases were patients who had at least two main signs including diarrhea (at least three times per day) and vomiting or fever or those who recorded diarrhea or gastrointestinal symptoms referring to Farabi district hospital. It is noteworthy that a case of diarrhea was defined as  $\geq 3$  per loose stool in the last 24 hours.<sup>4</sup> On the other hand, controls were native healthy non-diarrheal subjects without any digestive disorders or disease, who were selected from among the neighbors of the same village at the same period of the outbreak for age and sex-group matched cases. Group matching was used for adjusting potential confounder's sex and age. In this matching, subjects were divided into three age groups, and in each age group and sex, the same number of subjects were selected from each case and control. As regards gastrointestinal disease, infants were less than one year and adults were more than 70 years and subjects who disagreed to participate were excluded from the study.

### Data Collection

A researcher-made questionnaire was developed and assessed according to the World Health Organization (WHO) guideline.<sup>8</sup> This questionnaire focused on the



**Figure 1.** The diarrhea epidemic curve, Idahluy-e Bozorg, Malekan, East Azerbaijan, Iran, on Jan 2018.

drinking water supply as the probable source of the outbreak, it also included questions regarding other potential risk factors and exposures. Furthermore, the risk exposures investigated through this questionnaire consisted of demographic characters, clinical features, drinking water source, toilet hygiene, and handwashing after using the toilet (based on the WHO guideline, washing with soap or detergent for at least 10-15 seconds). Moreover, the environmental health of the village, the clinical symptoms of the patients, and the history of drinking and eating in the last 72 hours were considered as the other risk exposures that were evaluated using the questionnaire. The questionnaires were completed by trained interviewers (CHW) for notified cases and healthy controls through door to door surveys.

Similarly, the gathered data were checked for the quality and completeness of the responses before entering a database.

#### Laboratory Diagnosis of Etiological Agent of the Outbreak

After receiving the outbreak urgent report in the preliminary phase of the outbreak investigation, the water samples were collected in sterilized glass bottles from various sources of drinking water in the village such as NDW, well water, and other water sources. To identify possible bacterial contamination, total bacterial count and a fecal coli were obtained to determine fecal contamination. The fresh stool samples (for at least less than 1 hour) and fecal swabs were collected in Carry-Blair transport media and then brought to health network laboratory center, followed by testing the samples for detection. Histolytica trophozoite by stool moist lam since a large number of patients had bloody diarrhea. Additionally, other microbiological analyses such as *Vibrio cholerae* and *Escherichia coli* were performed according to the standard protocol. Moreover, the suspected consumed food samples were collected from patients and sent to the central laboratory of Tabriz University of Medical Sciences for microbial and biochemical tests while maintaining the cold chain (a temperature of 2-8°C).

#### Environmental Investigation

Likewise, the researchers performed an environmental survey to identify the following infection risk factors:

- Water supply source condition;
- The possibility of water contamination;
- Well and river water;
- Residual chlorine in the last 30 days in the village NDW;
- The number of days for the interruption of the drinking water of the village;
- The existence of fractures in NDW;
- The agriculture and vegetables water source;
- Household wastewater disposal;
- Village sewage disposal.

#### Statistical Analysis

Data were analyzed by SPSS (version 19.0, Chicago, IL, USA). In addition, the Kolmogorov-Smirnov test was applied to check data normality. The Chi-square test and independent *t* test were also utilized to assess dichotomous and continuous variables among the cases and controls. Further, the Mann-Whitney and Kruskal-Wallis tests were used for nonparametric variables. Finally, univariate and multiple logistic regressions were employed to estimate the adjusted odds ratio with a 95% confidence interval for the risk exposures and diarrhea. In all tests, the significance level and the confidence interval were  $P < 0.05$  and 95%, respectively.

#### Results

##### The Outbreak Description (i.e., Person, Time, and Place)

At the end of the outbreak, 250 diarrhea cases were identified during January 19-31, 2018 in Idahluy-e Bozorg village. Totally, 51.2% of the cases were female and the age group of 15-29 had the highest frequency (mode). Similarly, almost 70% of diarrhea cases had farming or farming-related occupation and more than 99% of them were native residents. About 76.8% of cases had a primary school degree of education or were poorly-educated as well (Tables 1 and 2).

The total population of the village was at risk. The outbreak type was recognized as a continuous common-source epidemic based on the incubation period of amebiasis (1-3 days) and the pattern of the outbreak curve in relation to the disease sums by the days of the outbreak (Figure 2). Furthermore, the peak day of the incidence was January 21, 2018, with 59 diarrhea cases. Eventually, the case fatality rate and attack rate were reported to be zero and 45.7% (250/547), respectively.

**Table 1.** Person, Time, and the Place of Individuals Involved in the Outbreak

Variable		No.	%
Gender	Female	128	51.2
	Male	122	48.8
Age	Mean ± SD	29±6.76	
	≤14	67	26.8
	15-29	98	39.2
	≥30	85	34
Occupation	Farming related	173	69.2
	Not-farming	77	30.8
Educational level	Primary school	192	76.8
	Secondary school	45	18
	High school or academic	13	5.2
Native resident	Yes	248	99.2
	No	2	0.8
Total		250	100

**Table 2.** Results of Organisms Detected in Stool Samples

Organism	No. (%)
<i>E. histolytica</i> (trophozoite)	24 (66.66)
<i>E. coli</i>	7 (19.44)
Joint <i>E. histolytica</i> (trophozoite) and <i>E. coli</i>	5 (13.88)
Shigellosis	0
<i>Salmonella</i>	0
<i>Vibrio-cholera</i>	0
None	0
Total	36/36

### Laboratory Results

Based on the findings, out of the total of 36 tested stool samples, the common organisms/ parasites were detected as 24 (66.66%) *E. histolytica* (trophozoite) without any *Vibrio-cholera* (Table 3).

Likewise, from among the total six water samples (four samples of NDW and two samples of well water), coli forms were observed in all four samples of NDW, but no ones were observed in the two samples of well water. Moreover, no organisms were found in the two specimens of consumed foods by patients.

### Results of Case-Control Design

According to the findings, the most common symptoms were diarrhea and abdominal pain (88.7%) among all patients. Table 4 presents the comparison between case and control groups for demographic characters and the other risk factors with the odds of amebiasis. From 62 cases of amebiasis, 53.22% were female and the most affected age group consisted of 1-14-year-old cases (almost 20%). There was a statistically positive significant relationship between case and control groups regarding NDW ( $P=0.001$ ). Additionally, a protective association was observed between well water consumption ( $P=0.003$ ), boiling water ( $P=0.003$ ), toilet sanitary ( $P=0.025$ ), and complete handwashing after using the toilet ( $P=0.017$ ).

After adjusting for the potential confounders by multiple logistic regression, it was indicated that NDW consumption increased the odds of disease 16.56 times (16.56, 95% CI=4.14-64.87) whereas well water and boiling water consumption had a protective association and decreased the odds of amebiasis (1- OR) 81% and (1- OR) 66%, respectively. Related data are provided in Table 5.

### Results of Environmental Investigation

Our environmental door to door survey, along with investigations about indigenous residents and local water supply company revealed that the source and repository of the outbreak was human sewage and outbreak happened due to the entrance of human sewage into the drinking water. Our environmental and field investigations

**Table 3.** Distribution of Clinical Symptoms of All Cases in the Outbreak

Symptom	No. (%)
Diarrhea	250 (100)
Blood diarrhea	47 (18.80)
Abdominal pain	168 (67.20)
Vomiting	134 (53.60)
Fever	107 (42.80)
Others	76 (30.40)

demonstrated that the pipe of village drinking water and human sewage were in contact and adjacent together in the underground. In addition, several fractures were detected in the line of NDW. Furthermore, the village VDW was experiencing an outage approximately nine times (at least three hours per time) in January 2018. Based on the findings, the residual chlorine was less than 0.2 ppm in the village NDW in the last 20 days.

### Discussion

In general, a continuous common source epidemic was revealed considering our study results, the incubation period of amebiasis, and the pattern of outbreak. Further, based on the findings of the outbreak investigation and the tested stool samples, *E. histolytica* was detected as the etiologic agent of the outbreak. Furthermore, human sewage was found as the source and repository of the outbreak. Applying multiple logistic regression after adjusting for sex and age groups, the results of the case-control study analysis represented that the NDW consumption in the last 72 hours was highly associated with the outbreak agent and increased odds of the diarrhea risk and thus confirmed our hypothesis concerning the source of the outbreak. Given that the source and the main reservoir of *E. histolytica* were patients, water contamination with human sewage was found to be the most probable agent of the outbreak. Therefore, our environmental survey had the same results and approved the hypothesis. Similarly, several fractures were observed in the line of NDW and the source of infection was detected due to the contamination of the underground NDW by human pipe sewage. The village NDW and human pipe sewage were in contact and adjacent together as well. The final surveys revealed that the entrance of human sewage from the village human network sewage into the NDW was the source of infection. The exploration of the regional HS and local water supply company confirmed this event. The outbreak was silent and ended without deaths after closure and the resolving of NDW contamination and pipe fractures.

In agreement with our study results, the newest diarrheal disease outbreak was reported in Gaidatar village of Rautahat district, Nepal in 2019 by transmission through

the drinking water sources contaminated with fecal matters from their sewage (drainage) system.<sup>10</sup> Many waterborne outbreaks occur in Iran per year but the outbreaks are rare due to NDW contamination by human pipe sewage with *E. histolytica*. Based on the results of previous studies, the diarrhea outbreak was related to drinking water in Pardis, Iran, which is in line with our findings, as well as with those of Masumi-Asl et al.<sup>11-14</sup>

Likewise, an *E. histolytica* outbreak was detected in 730 affected students in a school in Taiwan.<sup>15</sup> This infection

was originated from the contamination of underground well water by sewage from a toilet. Another amebiasis outbreak was reported in Tbilisi, the Republic of Georgia, indicating that drinking water was the source of infection.<sup>16</sup> Correspondingly, *E. histolytica* has recently been recognized as an emerging sexually transmissible pathogen in men who have sex with men, which was observed in a Japanese institution for individuals with mental retardation.<sup>17,18</sup>

According to some studies, the amoeba is considered as one of the most common pollutant parasites in consumed

**Table 4.** Demographic and Risk Factors of Case and Control Groups with the Odds of Amebiasis in the Outbreak

Variables		No. of Control (%)	No. of Case (%)	P Value
Gender*	Female	31 (50)	33 (53.22)	0.721
	Male	31 (50)	29 (46.77)	
Age*	≤ 14	26 (41.93)	24 (38.71)	0.459
	15-30	16 (25.80)	13 (20.96)	
	≥ 30	20 (32.26)	25 (40.32)	
Occupation	Farming related	33 (53.22)	35 (56.45)	0.243
	Not-farming	29 (46.77)	27 (43.54)	
Marital status	Married	32 (52.23)	34 (54.83)	0.285
	Single	29 (46.77)	26 (41.93)	
	Widow and divorced	1 (1.61)	2 (3.23)	
Educational level	Primary school	27 (43.54)	22 (41.93)	0.247
	Secondary school	30 (48.38)	36 (58.06)	
	High school and academic	5 (8.06)	4 (6.45)	
Toilet status	Sanitary	32 (51.61)	22 (35.48)	0.025
	Improved	13 (20.97)	12 (19.35)	
	Insanitary	17 (27.42)	28 (45.16)	
Handwashing after toilet <sup>a</sup>	Complete	32 (51.61)	19 (30.64)	0.017
	Incomplete	30 (48.38)	43 (69.35)	
Domestic waste disposal	Sanitary	36 (58.06)	34 (54.83)	0.717
	Insanitary	26 (41.93)	28 (45.16)	
Wastewater disposal	Sanitary	45 (72.58)	40 (64.51)	0.334
	Insanitary	17 (27.42)	22 (35.48)	
Network drinking water**	Yes	35 (56.45)	59 (95.16)	0.001
	No	27 (43.54)	3 (4.83)	
Well water**	Yes	31 (50)	14 (22.58)	0.003
	No	31 (50)	48 (77.41)	
Fountain water**	Yes	10 (16.12)	5 (8.06)	0.165
	No	52 (83.87)	57 (91.93)	
External water***	Yes	15 (24.19)	9 (14.51)	0.256
	No	47 (78.33)	53 (85.43)	
Boiling water**	Yes	27 (43.54)	12 (19.35)	0.003
	No	35 (56.45)	50 (80.45)	
Syrup or Juice**	Yes	28 (45.16)	23 (37.09)	0.321
	No	34 (54.84)	39 (62.90)	
Milk**	Yes	36 (58.06)	28 (45.16)	0.208
	No	26 (72.57)	34 (54.38)	
Dough**	Yes	29 (46.77)	21 (33.87)	0.141
	No	33 (53.22)	41 (66.13)	
Ice-cream	Yes	18 (27.42)	21 (33.87)	0.284
	No	44 (70.97)	41 (66.12)	
<b>Total</b>		<b>62</b>	<b>62</b>	

Note. \*Matched variables; \*\*In the last of 72 hours; \*\*\*Used the water of the other villages or city water for drinking; <sup>a</sup>Used the soap or detergent for at least 10-15 seconds.

**Table 5.** Crude and Adjusted OR and 95% CI for Amebiasis by Multiple Logistic Regressions\* in Diarrhea Outbreak

Variables	No. of Controls (%)	No. of Cases (%)	Crude OR (95% CI)	Adjusted OR (95% CI)
<b>Toilet status</b>				
Sanitary	32 (51.61)	22 (35.48)	Ref	Ref
Improved	13 (20.96)	12 (19.35)	1.34 (0.51-5.39)	2.58 (0.76-8.74)
<i>P</i> value			0.541	0.125
Insanitary	17 (27.41)	28 (45.16)	2.39 (1.07-5.39)	3.86 (1.37-10.91)
<i>P</i> value			0.035	0.011
<b>Network drinking water</b>				
Yes	35 (56.45)	59 (95.62)		
No	27 (43.54)	3 (4.83)	15.17 (4.28-52.69)	16.56 (4.14-64.87)
<i>P</i> value			0.001	0.001
<b>Well water</b>				
Yes	31 (50)	14 (22.58)		
No	31 (50)	48 (77.41)	0.29 (0.13-0.63)	0.19 (0.07-0.51)
<i>P</i> value			0.003	0.001
<b>Boiling water</b>				
Yes	27 (43.54)	12 (19.35)		
No	35 (56.45)	50 (80.64)	0.311 (0.13-0.69)	0.34 (0.12-0.92)
<i>P</i> value			0.001	0.033
<b>Total</b>	<b>62</b>	<b>62</b>		

Note. OR: Odds ratio; CI: Confidence interval; \*Adjusted for sex and age.

vegetables.<sup>19,20</sup> In addition, the case reports approved liver and kidney complications by *E. histolytica*. The average prevalence of *E. histolytica* infection among Iranian population was reported about 1% in a systematic review and meta-analysis study conducted by Haghghi et al, while a higher prevalence (more than 1%) was observed with lower human development index provinces.<sup>7</sup>

*Entamoeba histolytica* infection is prevalent in developing countries. Several studies were reported from around the world in relation to amebiasis infection and outbreaks.<sup>21-26</sup>

Infectious disease outbreaks have emergency features and characters, and they may even occur in developed countries. In a systematic review study of waterborne disease outbreaks in Canada and the United States,<sup>27</sup> the failure of an existing water treatment system and the lack of water treatment were detected as the most leading causes of the outbreaks. Needless to say that most of the outbreaks occurred in summer months, which is consistent with the findings of our study.

Well water consumption is one of the most harmful risk factors or the source of infection in diarrhea outbreaks<sup>14,15</sup> whereas in the present study, subjects who consumed well water had lower problems, and well water played a protective role. It is because, in our study, NDW was contaminated by human sewage and was detected as the source of the outbreak. Another protective factor in the current study was the boiling water which can cause the death of amoeba cysts. Subjects who consumed the boiling

water had lower odds of amebiasis risk. Moreover, in multiple logistic regressions analysis after controlling for potential confounders, boiling water revealed a statistically significant relationship with the decreased odds of diarrhea. Besides, external water consumption from city water or the drinking water of the other villages had protective effects but were not statistically significant.

Based on the results of our environmental survey, the village NDW experienced outage approximately nine times (per time at least 3 hours) in January 2018 (during the outbreak) and the onset residual chlorine in NDW was less than 0.2 ppm in the last 20 days before the outbreak. Another finding pertained to several fractures in the village pipeline of NDW. Hence, these factors forced village people to consume well water and water for drinking from other villages or cities. Households or individuals who consumed this water were not affected by such factors. In our study, the frequency of subjects who used well water or boiled water for drinking was more than that of the case group.

*Entamoeba histolytica* infection is prevalent in poor sanitary conditions, low human development index, and tropical areas with a high population density. Additionally, *E. histolytica* infection has a very strong relationship with the insanitary status of the toilets.<sup>28</sup> The results of our study indicated that the toilet conditions of the cases were significantly insanitary than that of the controls, therefore, this relationship was reported statistically significant in

the final analysis. Furthermore, in cases group, complete handwashing after using the toilet was less frequently practiced in comparison to the control group, which increased the odds of amebiasis. It is assumed that toilet insanitary status and incomplete handwashing after using the toilet were related to each other thus, it is likely that part of the transmission cycle in the outbreak was pertinent to patients who suffered from insanitary toilet condition or inadequate hand washing. The findings of global and community, as well as hospital-based studies were also in agreement with our findings.<sup>10-14,29-31</sup>

In the present study, most patients (42%) were in  $\geq 14$  age groups. Other studies also found that children were more susceptible and infected, especially in the outbreaks.<sup>32,33</sup>

### Limitations

The present study has several limitations. Diarrhea cases were not all confirmed by the laboratory; nevertheless, standard diarrhea definition was used for patients' selection. In addition, the sudden nature of the outbreak has been deprived of the opportunity to select more patients, stool samples, and exposure information in relation to the source of the outbreak and etiologic agent.

### Conclusion

Overall, *E. histolytica* was found to be the etiologic agent of the continuous common source of the outbreak. Further, the source of infection originated from the contamination of underground NDW by human pipe sewage. HS readiness, along with timely recognition and immediate response to the outbreaks, can prevent disease infection and deaths. In this outbreak, several factors accelerated the outbreak episode, including the erosion of water supply network, the frequent disconnection of the water network, the undesirable residual chlorine of drinking water, poor sanitary conditions, and finally, climate warming. Accordingly, it is highly recommended to perform additional monitoring of water systems, particularly during the summer. HS and Water Supply Company should also be in complete readiness to cope with these outbreaks.

### Ethical Approval

This study was approved by Malekan Health Network Ethics Committee under the ethical number of 1397/4530/3D and informed consent was obtained from all study subjects. This research is part of the legal and administrative task of controlling an epidemic by health network. A case-control study also is just one part of this process. So there was no need to get a code of ethics from the university.

### Conflict of Interest Disclosures

None.

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