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ORIGINAL ARTICLE

The First Outbreak of Giardiasis with Drinking Water in Korea

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Abstract
Objectives: To identify the pathogen of the diarrhea outbreak in a village in Jeollabuk province in Korea in April 2010.
Methods: DNA extraction was performed from the 120 L of collected water, which was centrifuged at 10,000 x g for 30 min. PCR reactions were conducted in a total of 25 ul, which included PCR premix (GenDEPOT, Barker, TX, USA), 2 ul (~100 ng) of extracted DNA, and 10 pmol of each primer.
Results: Nine people out of 25 had a symptom of abdominal pain accompanied by diarrhea after they used stored valley water in a water tank as a provisional water supply source without chlorine sterilization. Among them *Giardia lamblia* was detected in fecal samples of 7 people using the polymerase chain reaction method. Although *G. lamblia* was also detected from water provided by the provisional water supply system stored in the water tank and used as drinking water, it was not detected in the water tank itself. This water-borne outbreak is considered to have occurred when the provisional water supply tube was destroyed under a building construction and contaminated by *G. lamblia*, but its precise cause has not been clarified.
Conclusion: This outbreak resulting from *G. lamblia* is very meaningful as the first outbreak of an infection by a water-borne parasite in Korea.

1. Introduction

Waterborne infections are enteric diseases that are called gastroenteritis in advanced countries. These are very frequent diseases and various reports suggest that their outbreaks in adults occur at least two times a year [1–6]. One of the most noteworthy characteristics of *Giardia*, a waterborne parasite that causes giardiasis, is that its

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eradication rate by sterilization is very low compared with other pathogens. A typical example of its outbreak occurred in Bergen (Norway) in 2004, when 2400 patients were infected and most outbreaks were caused by inflow of ranch wastewater, defective drinking water pipes, and inadequate water purification treatment [7,8].

In May 2010, cases of acute diarrhea were reported in Korea. Consequently, environmental research on the outbreak and epidemiological research on the patients with diarrhea were conducted. This report presents the outbreak of the first waterborne infection caused by the parasite *Giardia lamblia*.

2. Materials and Methods

2.1. DNA extraction

DNA was extracted from the 120 L of collected water which was centrifuged at 10,000 x g for 30 min. The supernatant was completely removed, and DNA was extracted from the precipitate using a QIAamp DNA stool mini kit (QIAGEN, Hilden, Germany). After centrifugation, the pellet precipitate was transferred to a 2 ml Eppendorf tube and completely dissolved in 1.4 ml of the ASL buffer from the kit. The solution was subjected to five freeze/thaw iterations and then incubated at 95 °C for 10 min. The remaining DNA extraction steps followed the manufacturer's instructions. The extracted DNAs were used as templates in the primary PCR reactions.

2.2. PCR reaction

PCR reactions were conducted in a total of 25 ul, which included PCR premix (GenDEPOT, Barker, TX, USA), 2 ul (∼100 ng) of extracted DNA, and 10 pmol of each primer (Table 1). The PCR reactions were conducted with a G-STORM apparatus (Gene Technologies, England). The DNAs were denatured for 7 min at 94 °C, followed by 35 cycles of 60 sec at 94 °C, 60 sec at 60 °C or 52 °C, and 90 sec at 72 °C. Finally, reactions were incubated for 10 min at 72 °C. Nested PCR was conducted with 1 ul of the primary PCR product as template. After the nested PCR, the final product was electrophoresed in a 2% agarose gel and stained with ethidium bromide. Bands were visualized under ultraviolet light, and photos were taken using a gel documentation system (Syngene, Cambridge, UK).

3. Results

People in the villages of Baekun-dong, Baekun-meon, Jinan-gun, and Jeollabuk-do use ground water and provisionally supplied water. Seven of the eight households (25 people) that used provisionally supplied water complained of abdominal pain accompanied by diarrhea from around 8:00 AM on the morning of April 12, 2010. The village chief reported their case to the local health district board, which sent a local epidemiological investigator for investigation. The investigator asked the 25 people to fill out a questionnaire. It was verified that they had not eaten meals together. Approximately 1500 L of the water from the common provisional water supply system was then collected and analyzed by polymerase chain reaction (PCR). Fecal samples of the 25 people in eight households (patient group: nine people; asymptomatic control group: 16 people), who were using the provisionally supplied water, were collected. Those who had at least one of the following symptoms were considered patients: vomiting, abdominal pain, febricity, chillness, or a sense of residual stool. Jeollabuk-do Institute of Health and Environment Research conducted the PCR test to detect the presence of *G. lamblia*, while the Korea Centers for Disease Control and Prevention conducted a confirmation test.

Out of the 25 who used provisionally supplied water, three among the nine in the patient group and four among the 16 in the control group tested positive for *G. lamblia* infection, according to the results of PCR analysis targeting the *β-giardin* gene. A sequence analysis of the *β-giardin* gene was also positive for *G. lamblia* infection. The incidence rate was 36% with nine symptomatic patients out of the 25 who used the provisional water supply system being exposed to risk factors. They mostly had diarrhea and abdominal pain, followed by a sense of residual stool and vomiting, and they had diarrhea a maximum of six times (Figure 1).

4. Discussion

The causative pathogen of this mass diarrhea was confirmed to be *G. lamblia* and this was the first

Table 1. Primer sets used for detection of *Giardia lamblia*

Target gene	Primer name	Sequence	PCR product size (bp)	Tm (°C)
β-giardin Accession no. M36728	G376A F	5′-CCA TCC ATA ACG ACG CCA TCG CGG CTC TC-3′	415	60
	GGR789-809B R	5′-GGC GCT TAG TGC TTT GTG ACC-3′		
	G376B F	5′-CGA CGC CAT CGC GGC TCT CAG GAA GGA GG-3′	374	60
	G759A R	5′-CGC CCT GGA TCT TCG AGA CGA CGT CCT-3′		

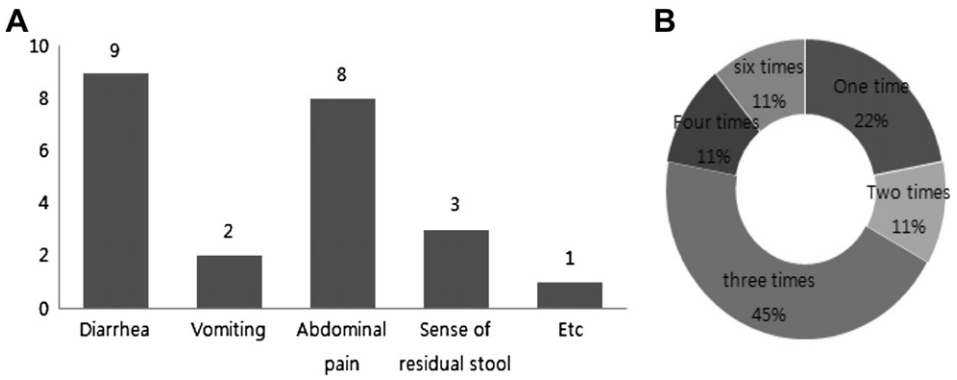

Figure 1. (A) Main symptoms in infected patients and their frequencies; (B) frequency of diarrhea in infected patients/day.

epidemiological investigation of mass diarrhea caused by this parasite. The Centers for Disease Control and Prevention investigated the rates of infections caused by waterborne protozoans (*G. lamblia*, *Cryptosporidium parvum*, and *Entamoeba histolytica*) in 96 hospitals and clinics for 3 years from 2004 to 2006. The overall positive rate stood at approximately 0.17%; detection of *G. lamblia* among these parasites accounted for 55% and its outbreak was most frequent during the period from April to May [9]. It is to be noted that the outbreak presented in this report also occurred in April. Cheun et al (2010) reported *G. lamblia* infection with viral infection in patients, and the mixed infection rate with norovirus was 4.7%. Tests to identify 10 causative bacteria of food poisoning and norovirus were performed on fecal samples of patients with diarrhea. While three patients tested positive for norovirus, the other patients were negative for all the other bacteria. Giardiasis infection is more frequent in developing countries than in developed countries and in Europe approximately 3−14% of patients with human immunodeficiency virus (HIV) infection/acquired immunodeficiency syndrome were reported to be positive for the parasite [10]. In Korea, 1.5% of patients with HIV have been reported to be infected with *Giardia* [11] and genetic classification to Nash's group was conducted by separating the parasite from outpatients in 1999. However, genetic classification of *G. lamblia* was not carried out due to the shortage of its DNA in this study.

Because of this outbreak, provisional water supply was stopped, and public health education for residents on the safety of heating drinking water was conducted. Water to the households was supplied through Ginan-gun water tank trucks, while kitchens and rest rooms were chlorinated, quarantined, and disinfected. Eventually, *G. lamblia* was not detected. Sodium hypochlorite is only used to disinfect small-scale water supply systems such as provisionally supplied water or well water in Korea [12, 13]. This is because the pathogen is highly resistant to temperature, solar irradiation, sodium hypochlorite, and chlorine dioxide treatments on a large scale [14−17]. However, this outbreak was through the provisional water supply system using unsterilized valley water.

In Korea, the testing and treatment of diarrhea has been focused mainly on viruses and bacteria, and currently there is a lack of tests to diagnose diarrhea caused by protozoans. According to the 2009 water and foodborne epidemiology annual report [18], diseases from unknown pathogens accounted for 40% of infections and in most cases, diarrhea resulting from causative waterborne parasitic infections was suspected. Finally, this outbreak by *G. lamblia* suggests the importance of continuous monitoring of waterborne and foodborne parasites in order to prevent and control the spread of parasites.

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