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Ground water as the source of an outbreak of *Salmonella* Enteritidis

Ana Kovačić [a,*], Željko Huljev [b], Edita Sušić [b]

[a] Public Health Institute of Split and Dalmatia County, Split, Croatia
[b] Public Health Institute of Šibenik and Knin County, Šibenik, Croatia

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ABSTRACT

In September 2014, an outbreak of gastroenteritis was reported to the Public Health Institute of Šibenik and Knin County in Croatia. The outbreak occurred in the County center of Šibenik, a town with 50,000 inhabitants, and it lasted for 12 days. An epidemiological investigation suggested a nearby water spring as the source of the outbreak. Due to the temporary closure of the public water supply system, the inhabitants started to use untreated water from a nearby spring. Microbiological analysis revealed that the outbreak was caused by *Salmonella enterica* subsp. *enterica* serovar Enteritidis that was isolated from stool samples of the patients and ground water. The isolates were further analysed with pulsed-field gel electrophoresis using *Xba*I, which revealed an identical macrorestriction profile. Although 68 cases were reported, it was estimated that the actual number of affected persons was more than several hundred. In order to prevent further spread of disease, public advice was released immediately after the first epidemiological indication and a warning sign was placed at the incriminated water source, after microbiological confirmation. It is necessary to regularly monitor microbiological quality of ground water especially in urban areas and provide adequate education and awareness to the inhabitants regarding the risk of using untreated ground water.

1. Introduction

Salmonella is one of the most important causes of gastroenteritis worldwide. The symptoms of infection include fever, abdominal pain, diarrhoea, nausea, and sometimes vomiting. They usually appear 12–72 h after infection and last for 4–7 days, without any consequences for most patients [1]. The outbreaks usually occur due to the consumption of contaminated food and water [2–4]. Various sources of *Salmonella* spp. are found in the environment and they include livestock, wildlife, and poultry, but they also abound in natural waters [2,5]. More than 2500 *Salmonella* serovars are found in environmental samples where they can persist for long periods [2,3,5]. It has also been found that genotypically related strains can be isolated from water and wildlife samples, which suggests that they could be reservoirs for the dissemination of *Salmonella* in the environment [5]. Moreover, natural waters play an important role as vehicles of transmission of these microorganisms. Water contamination and outbreaks with nontyphoidal serovars are rarely reported, despite the fact that they are often present in surface waters [3,6–8]. Almost all outbreaks in developed countries are related to the use of untreated or inadequately treated water [3]. It has been estimated that ~1.1 billion people globally drink unsafe water and as much as 88% of diarrhoeal disease in the world is attributable to unsafe water, sanitation, and hygiene [9]. Surveys of waterborne outbreaks of *Salmonella* are mainly conducted in developed countries where small numbers of such cases are reported. In the USA, there were 15 cases of nontyphoidal *Salmonella* outbreaks caused by drinking water from 1971 to 2000 and none were registered in the period from 2000 to 2012 [10–12]. However, *Salmonella* was not associated with waterborne outbreaks in England and Wales from 1992 to 2003 [12]. Contaminated waters are often used for irrigation or the washing of food products, so even though the true incidence of waterborne outbreaks caused by this microorganism is probably greater, it is not recognised as a waterborne disease [3]. The goal of our study was to find out the cause of the outbreak and to determine its source.

On September 12, 2014 a water supply system of the Croatian town of Šibenik (Fig. 1), which supplies ~80,000 inhabitants, was temporarily closed due to extreme heavy rainfall and fear of possible pollution. That same day, the residents were warned that water from the pipes was not fit for human consumption and they were supplied with freshwater tanks. The first patients suffering from

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* Corresponding author at: Public Health Institute of Split and Dalmatia County, Vukovarska 46, 21000 Split, Croatia.
E-mail address: ana.kovacic@st.t-com.hr (A. Kovačić).

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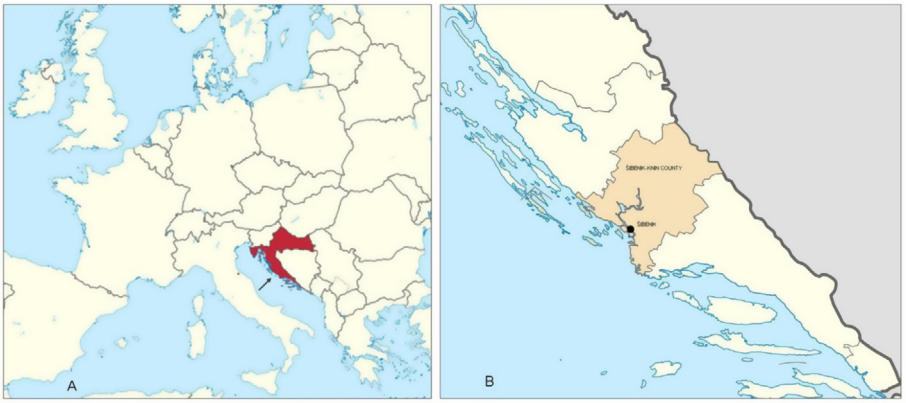

Fig. 1. (A) Position of Croatia in Europe. (B) Šibenik-Knin County and location of the study.

gastroenteritis contacted their family physicians on September 18, 2014. Five days later, an epidemiologist from the regional Public Health Institute was informed, by the microbiological laboratory, of a higher than usual number of *Salmonella enterica* subsp. *enterica* serovar Enteritidis (*S.* Enteritidis) in stool samples. By the end of the following week >68 cases were registered. The first epidemiological investigation revealed that two of the hospitalised patients consumed water from the ground water source, situated ~15 km from the town of Šibenik (Fig. 2). The ground water from the source was sampled aseptically and sent to the microbiological laboratory for analysis, together with the water sample from the canister of one of the patients. The patient kept water from the source in the canister and consumed it repeatedly.

2. Materials and methods

2.1. Epidemiological data

Hypothesis-generating interviews conducted with 36 cases suggested that consumption of ground water from the source was the most important risk factor associated with disease. A case was defined as a person suffering from acute gastroenteritis with onset between September 12, 2014 and October 16, 2014 and with a stool sample positive for *S.* Enteritidis. All cases were interviewed by an epidemiologist in order to obtain standardised epidemiological information. The interview included personal information, data regarding illness, travel history, and all exposure-related variables (dietary habits, recreational activities, and pets). Detailed information was also collected on any water consumption including whether it was consumed from ground water springs or water bottles that were filled at the spring. The surrounding area was explored by epidemiologists and local hunters, searching for any animal traces or biological waste.

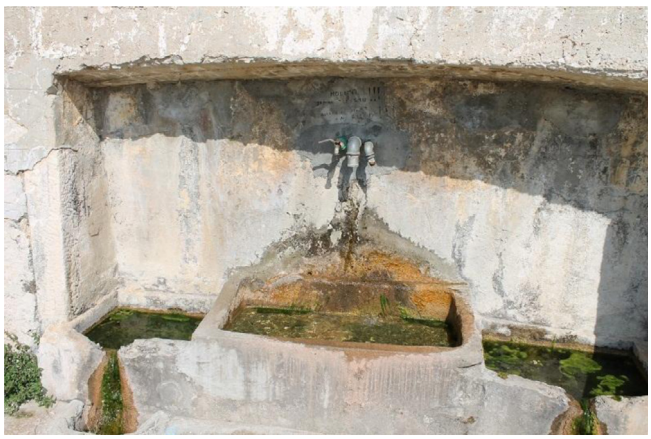

Fig. 2. Unchlorinated ground water source of the outbreak.

2.2. Microbiological analysis of stool samples

Stool samples were inoculated in Selenite Broth and xylose lysine deoxycholate (XLD) agar (Becton Dickinson, Franklin Lakes, NJ, USA) and incubated at 37 °C for 24–48 h in air atmospheric. Suspicious nonlactose fermenting and H_2S-positive colonies (red colonies with black pigmentation and pink halo) were biochemically tested and identified with an automated VITEK 2 Compact System (bioMerieux, l'Etoile, France). Such colonies were also serotyped by group- and type-specific antibodies that react with O and H determinants (BioRad, Paris, France). Susceptibility of the strains to ampicillin/amoxicillin, amoxicillin–clavulanic acid, ceftazidime, ceftriaxone, pefloxacin (screen for ciprofloxacin resistance), chloramphenicol, and trimethoprim + sulfamethoxazole was determined using a disc diffusion test (Mast Diagnostics, Bootle, Merseyside, UK). Our laboratory participates in the United Kingdom National External Quality Assessment Schemes for general bacteriology and antimicrobial susceptibility. The results of disc diffusion tests were interpreted according to the European Committee on Antimicrobial Susceptibility Testing criteria (version 4.0, 2014) and Committee for Antibiotic Resistance Surveillance in Croatia [12–14].

2.3. Microbiological analysis of water

Two samples of water, one from the spring and the other from the canister of the patient, were tested for *Salmonella* spp. according to International Organisation for Standardisation 19250:2013 [15]. Samples of water from the spring were taken in a 1-L sterile bottle and the patients brought their canisters with water that were filled at the same place. After inoculation in nonselective and selective broths, samples were transferred onto XLD agar and Brilliance Salmonella Agar (Oxoid, Basingstoke, UK). All suspicious (nonlactose fermenting and H_2S positive) colonies that appeared as red with black centers on XLD or purple on Brilliance Salmonella Agar were biochemically tested using API 20E (bioMerieux). The isolates were serotyped and further tested for susceptibility as described above for colonies from stool samples.

2.4. Pulsed-field gel electrophoresis analysis

In order to determine their clonal relationship, 13 isolates of *S.* Enteritidis from stool samples and one from water were analysed