



Article

Pediatric Epidemic of *Salmonella enterica* Serovar Typhimurium in the Area of L'Aquila, Italy, Four Years after a Catastrophic Earthquake

Giovanni Nigro ^{1,*}, Gabriella Bottone ¹, Daniela Maiorani ¹, Fabiana Trombatore ¹,
Silvana Falasca ² and Gianfranco Bruno ²

¹ Pediatric Unit, University of L'Aquila, San Salvatore Hospital, L'Aquila 67100, Italy; gabriellabottone@gmail.com (G.B.); daniela.maiorani82@gmail.com (D.M.); fabiana_t@alice.it (F.T.)

² Department of Clinical Pathology, San Salvatore Hospital, L'Aquila 67100, Italy; silvana.falasca@alice.it (S.F.); supergiarf@gmail.com (G.B.)

* Correspondence: nigrogio@libero.it; Tel./Fax: +39-068-845-089

Academic Editor: Karin Nielsen-Saines

Received: 22 February 2016; Accepted: 29 April 2016; Published: 6 May 2016

Abstract: *Background:* A *Salmonella enterica* epidemic occurred in children of the area of L'Aquila (Central Italy, Abruzzo region) between June 2013 and October 2014, four years after the catastrophic earthquake of 6 April 2009. *Methods:* Clinical and laboratory data were collected from hospitalized and ambulatory children. Routine investigations for *Salmonella* infection were carried out on numerous alimentary matrices of animal origin and sampling sources for drinking water of the L'Aquila district, including pickup points of the two main aqueducts. *Results:* *Salmonella* infection occurred in 155 children (83 females: 53%), aged 1 to 15 years (mean 2.10). Of these, 44 children (28.4%) were hospitalized because of severe dehydration, electrolyte abnormalities, and fever resistant to oral antipyretic and antibiotic drugs. Three children (1.9%) were reinfected within four months after primary infection by the same *Salmonella* strain. Four children (2.6%), aged one to two years, were coinfecting by rotavirus. A seven-year old child had a concomitant right hip joint arthritis. The isolated strains, as confirmed in about the half of cases or probable/possible in the remaining ones, were identified as *S. enterica* serovar Typhimurium [4,5:i:-], monophasic variant. Aterno river, bordering the L'Aquila district, was recognized as the main responsible source for the contamination of local crops and vegetables derived from polluted crops. *Conclusions:* The high rate of hospitalized children underlines the emergence of a highly pathogenic *S. enterica* strain probably subsequent to the contamination of the spring water sources after geological changes occurred during the catastrophic earthquake.

Keywords: *Salmonella enterica* serovar typhimurium; *Salmonella* epidemic; *Salmonella* reinfection; water survey stations

1. Introduction

Salmonella enterica is one of the most common enteric pathogens of humans and animals, which causes over 90 million cases worldwide each year implying considerable illness and economic burden (in the United States >2 billion USD per year) [1]. *S. enterica* consists of >2500 serovars, of which *S. typhimurium* is the most ubiquitous in zoonotic reservoirs for human infection and the environment [2]. Over the past half century, the epidemiology of *S. typhimurium* has been characterized by successive waves of dominant multidrug-resistant clones [3]. During a 2005–2012 epidemic, comparative whole-genome sequencing and phylogenomic analysis of isolates from the United Kingdom and Italy showed high levels of genotypic variation of monophasic *S. typhimurium* affecting antigens, virulence factors, and resistance loci [4].

The monophasic variant of *S. typhimurium*, *S.* 4,[5],12:i:-, characterized by the antimicrobial resistance to Ampicillin, Streptomycin, Sulphonamide, and Tetracycline (pattern ASSuT) is emerging and extensively circulating in Denmark, Italy, United Kingdom, and Greece [4–6]. In Italy, *S. typhimurium* 4,[5],12:i:-, showed a dramatic increase since 2003, both in humans and in animals farmed for food production, particularly pigs and bovines [7]. Most serovars showed a marked seasonality, increasing over the summer months and peaking in August/September, which may be related to the parallel *Salmonella* shedding trend in animal hosts, and/or insufficient refrigeration and mishandling of foods during the warm months [8]. Non-typhoid *Salmonella* epidemics have never been associated with risk of consuming contaminated water following collapse of sewerage systems due to a devastating earthquake. We report about the *S. typhimurium* epidemic which occurred between June 2013 and October 2014 in children of the area of L'Aquila (Central Italy, Abruzzo region) four years after the catastrophic 6.3 magnitude earthquake of 6 April 2009.

2. Material and Methods

2.1. Patients

Over a period of three months (October–December 2013) the Epidemiological Unit of the San Salvatore hospital, L'Aquila, was alarmed because of an unusual number of children admitted with acute gastroenteritis or enterocolitis caused by *Salmonella* infections. To coordinate territorial investigation tasks, and to simplify sharing of the collected data, a multidisciplinary work-group was set up including the National Health Institute (Monitoring Network, EnterNet Italy), the Experimental Zoo-prophylactic Institute (Reference Regional Laboratory for Enterobacterial in, Veterinary Operation Centre for Epidemiology, Programming and Information), the ASL 1 Abruzzo (Department of Hygiene, Epidemiology and Public Health; and Department of Food and Nutrition Security). The following definitions were adopted: (a) Epidemic strain: isolated *Salmonella* strain; (b) Confirmed case: any subject resident in the area of L'Aquila from which the epidemic strain of *Salmonella* was isolated during the study period; (c) Suspected case: any subject resident in Abruzzo region and/or any subject who stayed in Abruzzo at least seven days before the onset of gastrointestinal symptoms; (d) Probable case: any subject resident in the area of L'Aquila, who was affected by diarrhea (at least three stools/24 h for three consecutive days) from June 2013 to October 2014.

2.2. Survey on Food Products and Environmental Matrices

The analyzed alimentary matrices included fresh sausage withdrawn from different food industries, home-made sausage, smoked and cured sausage withdrawn from one food industry, wild boar salami withdrawn from a food industry, ham, chicken/turkey or pork frankfurter withdrawn from kitchen serving school, chicken withdrawn from kitchens serving schools, liquid pasteurized eggs withdrawn from kitchens serving schools, fresh eggs, dry cured smoked ham and cured pork mayonnaise, and tap water from private houses. Numerous sampling sources for drinking water of the L'Aquila area were collected from the following sites: pickup points of the two main aqueducts, schools, main kitchens serving schools, private houses.

2.3. Microbiological Investigations

The isolated *Salmonella* strains were identified biochemically by Vitek 2 system (Biomerieux, Marcy-l'Etoile, France) and serologically by commercially available antisera (Statens Serum Institut, Copenhagen, Denmark) following the Kauffmann-White method. The strains were identified by multiplex polymerase chain reaction and typed by phage typing, pulsed-field gel electrophoresis (PFGE), and multiple-locus variable analysis (MLVA) following ECDC laboratory standard operating procedures [9,10]. Genomic DNA was extracted by Qiagen Easy Prep (Foster City, CA, USA), libraries were prepared using the Hi-Q sequencing kit, and sequencing was performed on a PGM Ion Torrent platform [6]. Raw reads were submitted to the SRA repository, and the samples were registered under

the project ID PRJNA266093. Reads were trimmed and assembled using a *de novo* workflow under the Oriane framework plus some specific python scripts [6].

3. Results

3.1. Clinical Features

The epidemic affected 155 children (83 females: 53%), all but two Italian, aged 1 to 15 years (mean 2.10). The distribution by age showed a prevalence of one to two year-old patients (Figure 1).

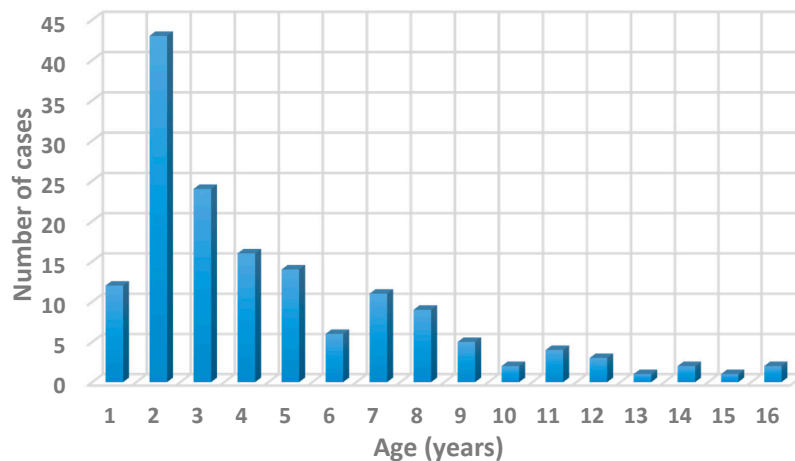


Figure 1. Distribution by age of 155 children with *Salmonella enterica* Typhimurium infection.

Forty-four of the infected children (28.4%) were hospitalized because of diarrhea, blood in almost half of them, severe dehydration, electrolyte abnormalities, vomiting, and fever resistant to oral antipyretic and antibiotic drugs. Three children (1.9%) were reinfected within four months after primary infection by the same *Salmonella* strain. Four children (2.6%), aged one to two years, were coinfecting by rotavirus. A seven-year old child had a concomitant right hip joint arthritis, the origin of which remained unknown because the synovial fluid (non-corporeal by ultrasound) was not taken. Figure 2 shows the distribution by months of the hospitalized children.

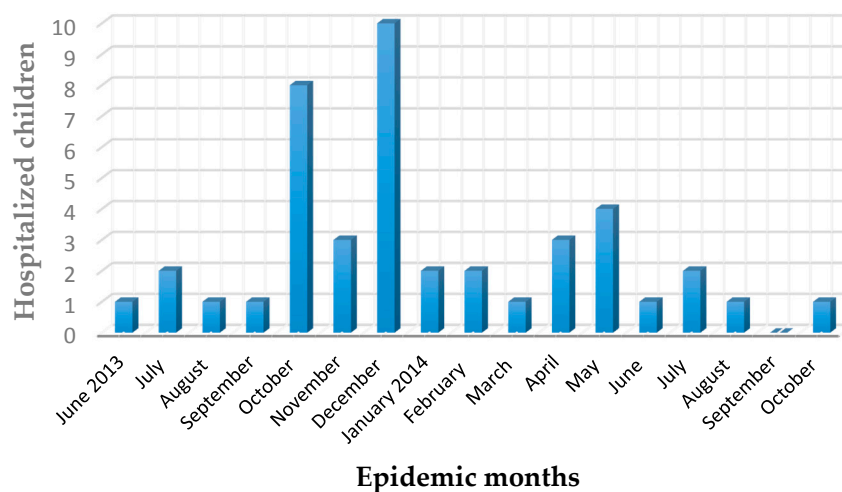


Figure 2. Distribution by months of 44 children with *Salmonella enterica* Typhimurium infection.

All the remaining children were followed up as outpatients since they had short-term diarrhea, which was associated with slight fever or vomiting in a minority of them (Table 1).

Table 1. Clinical findings in 155 children with *Salmonella enterica* Typhimurium infection hospitalized or followed up as outpatients.

Clinical Findings	Hospitalized Patients (44)		Outpatients (111)	
	No.	%	No.	%
Diarrhea	44	100	111	100
Bloody diarrhea	25	57	5	4.5
Fever	44	100	22	19.8
Dehydration	42	100	0	0
Electrolyte imbalance	16	36	0	0
Vomiting	28	64	7	6.3
Hip joint arthritis	1	2	0	0
Hospital stay (days)	4.1		–	

Mean laboratory results showed the occurrence of a slight neutrophilic leukocytosis with decreased levels of sodium (Table 2).

Table 2. Laboratory findings in 44 hospitalized children with *Salmonella enterica* Typhimurium infection, on admission.

Laboratory Findings	Range		Mean Values
	Min	Max	
Red blood cells ($\times \text{mm}^3$)	3.39	5.62	4.89
Hemoglobin (g/dL)	11.1	15.2	13.0
White blood cells ($\times \text{mm}^3$)	5.3	22.3	10.1
Neutrophils (%)	24.7	90.4	64.8
Lymphocytes (%)	7.8	58.4	23.6
Unclassified lymphocytes (%)	0.6	9.9	3.7
Platelets ($\times \text{mm}^3$)	213	836	330
Sodium (mEq/L)	126	142	133
Potassium (mEq/L)	3.4	5.1	4.0

During hospital stay, all children were given intravenous rehydration and intravenous antibiotic (amoxicilline-clavulanic acid in 40% and cephalosporins in 60% of cases) therapy. All children were discharged after confirmation of negative stool samples, and were given oral antibiotic therapy, to prevent reappearance of the symptoms and possible epidemic overspread.

3.2. Epidemiological and Bacteriological Investigations

S. typhimurium strains were isolated from samples of surface waters and sewage plants, all obtained in the area of L'Aquila. Serotyping of the isolated strains showed that all of them could be identified as *S. enterica* serovar *Typhimurium* [4,5:i:-], monophasic variant [6]. As reported by Orsini *et al.*, whole-genome sequencing was performed on 19 strains, which were chosen among clinical and environmental isolates because of their spatial-temporal distribution and familiar kinship, and three unrelated strains as outgroups. The genomic results, including numbers of coding sequences (CDSs), rRNAs, and tRNAs, N50 values and GenBank accession numbers, have been published [6]. On the basis of the maximum-likelihood phylogenetic tree derived from the Single Nucleotide Polymorphism matrix of sequenced strains, a close relationship between the isolates of water sources and isolates from patients was suggested [11].

Results of the survey showed that all the samples of drinking water and of chicken, veal meat, cooked and dry-cured hams, and eggs, which were the most consumed food one week before the clinical onset of infection, were negative. Other expositions to risk factors in the seven days before the onset of clinical symptoms of epidemic cases included school/nursery attendance, animal contact, exposition to symptomatic relatives, attending a banquet, traveling.

Starting from June 2014, the health authorities banned the use of surface waters for crop irrigation in the area of L'Aquila and decontaminating interventions were performed in water cleaning plants.

4. Discussion

S. typhimurium is the most reported serovar causing non-typhoidal salmonellosis in Italy since 2000, while *S. enteritidis* is still prevailing in other countries [7]. In immunocompetent individuals of high-income countries, infections by *S. typhimurium* mainly cause a self-limiting enterocolitis including vomiting, profuse watery diarrhea, and abdominal pain [12]. Invasive strains of non-typhoidal salmonellae can produce severe bloodstream infections in immunodepressed adults and malnourished children, as recently occurred during the outbreak caused by a new genotype of *S. typhimurium*, ST313, in the sub-Saharan Africa [13]. However, in both industrialized and developing countries, children aged one to six years have a higher risk of infection by *Salmonella* species [14]. During the epidemic occurred in the area of L'Aquila, the age risk was lowered to children less than two years of age. This elevated risk does not appear to be related to surveillance bias since clinical pathways (medical consultation, hospitalization, diagnostic tools) are the same for the age group of one to six years. Therefore, the higher risk of acquiring the disease may be due to age-related behavioral factors favoring greater susceptibility to the agent and risk of exposure to the vehicle of infection in domestic environment by cross contamination, particularly when the vehicle is a food for infants.

Contrary to previous *S. typhimurium* epidemic in Italy and other countries, the symptomatology was frequently severe in many children aged less than two years, and required hospitalization for rehydration and intravenous antibiotic therapy [1,7]. A five-year old boy also had a concomitant hip joint arthritis, which can occasionally occur during *S. enterica* infection [15].

Microbiological characteristics of the epidemic strain appear to be unusual, and the lack of correspondence with other strains in the database of human surveillance in Italy and in Europe does not allow the hypothesizing of animals as well as food as potential sources of infection. In fact, descriptive epidemiology suggests that the epidemic source may be represented by a locally produced and distributed vehicle. The potential sources fulfilling the epidemic scenario are the following: (a) long shelf life foods contaminated during the primary production process (e.g., food contaminated in the pre-harvest phase, stored and distributed within many months); (b) primarily contaminated short shelf life foods that steadily enter the market; (c) short shelf life foods that can be contaminated in the site of production environment and constantly enter the market of the Abruzzo region (e.g., food from animal origin such as meat, fruit and vegetables, dairy products). Moreover, the wide geographical origin of epidemic cases suggests that the contamination is not to be referred to a single cause but to a combination of different causes, probably of environmental origin.

Numerous reports underlined the high grade of contamination in the rivers of the Abruzzo region after the earthquake. Quality of the water of the Aterno river, bordering the L'Aquila district, was classified as "poor quality" and the river pollution was recognized as responsible for contamination of local crops irrigated by its water. Furthermore, as reported by Gordon *et al.* in Malawi, the local high rain rate subsequent to climatic changes lead to the same consequences by inducing the overflow of small effluents and subsequently the contamination of bordering crops [16]. Consequently, the source of infection could be related to ingestion of vegetable stocks prepared from manipulation of vegetables derived from polluted crops. In USA, several *Salmonella* outbreaks have been associated with irrigation waters as potential sources of contaminated food [17–19]. The occurrence of clinical cases due to the same *Salmonella* strain being isolated also in other regions only in patients who had travelled to L'Aquila could confirm the existence of unique environmental features in this area. Therefore, the initial cause of the epidemic spread may have been the geological change due to the catastrophic earthquake that occurred in the area of L'Aquila on 6 April 2009, and the subsequent contamination of the spring water sources following the system failure of the water cleaning plant collecting sewage. In 2007, Fujimoto *et al.* reported about the oil contamination of the Shinano river water after the Niigata Chuetsu earthquake occurred in 23 October 2004 [20]. To our knowledge, ours

is the first report about environmental water contamination by bacteriological pathogens following a catastrophic earthquake.

Finally, the emergence of a highly virulent strain of *S. typhimurium* could be related to the arrival to L'Aquila, since October 2011, of thousands of workers and their relatives from East European countries, who presumably hosted *Salmonella* strains different from those of people of L'Aquila. This observation may be supported by the hospitalization of only two non-Italian children, who were presumably in part immunized. The presence of different *S. typhimurium* strains during the epidemic may be supported by the occurrence of reinfections in three children.

5. Conclusions

In conclusion, our report highlights the emergence of a new highly pathogenic *Salmonella* strain causing a long term epidemic in the area of L'Aquila four years after a catastrophic event. To prevent serious epidemics consequent to geological reassessment and ecological changes, the water survey stations and sewage systems should be carefully monitored, and wider public health issues through structural changes in urban design should be addressed.

Acknowledgments: We are grateful to all doctors, nurses, and technicians of San Salvatore hospital who gave their contribution to the treatment of the infected children. We particularly thank the ward sister Maria Colaiuda and the director of the Department of Clinical Pathology Simonetta Santini. This study was conducted without financial support from any commercial source.

Author Contributions: Giovanni Nigro conceived and wrote the paper with contribution from Daniela Maiorani, Fabiana Trombatore, and Gabriella Bottone. Epidemiological and clinical data were collected and analyzed by Gabriella Bottone and Fabiana Trombatore. Silvana Falasca and Gianfranco Bruno performed the bacteriological examinations and contributed to the collection of laboratory data.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Majowicz, S.E.; Musto, J.; Scallan, E.; Angulo, F.J.; Kirk, M.; O'Brien, S.J.; Jones, T.F.; Fazil, A.; Hoekstra, R.M.; International collaboration on enteric disease "burden of illness" studies. The global burden of nontyphoidal *Salmonella* gastroenteritis. *Clin. Infect. Dis.* **2010**, *50*, 882–889. [[CrossRef](#)] [[PubMed](#)]
2. Wales, A.; Davies, R.H. Environmental aspects of *Salmonella*. In *Salmonella in Domestic Animals*, 2nd ed.; Barrow, P.A., Methner, U., Eds.; CAB International: Wallingford, UK, 2013; pp. 399–425.
3. Rabsch, W.; Trüepschuch, S.; Windhorst, D.; Gerlach, R.G. Typing phages and prophages of *Salmonella*. In *Salmonella, from Genome to Function*; Porwollik, S., Ed.; Caister Academic Press: Norfolk, UK, 2011; pp. 25–48.
4. Petrovska, L.; Mather, A.E.; AbuOun, M.; Branchu, P.; Harris, S.R.; Connor, T.; Hopkins, K.L.; Underwood, A.; Lettini, A.A.; Page, A.; *et al.* Microevolution of monophasic *Salmonella* Typhimurium during epidemic, United Kingdom, 2005–2010. *Emerg. Infect. Dis.* **2016**, *22*, 4. [[CrossRef](#)] [[PubMed](#)]
5. Trüpschuch, S.; Laverde Gomez, J.A.; Ediberidze, I.; Flieger, A.; Rabsch, W. Characterisation of multidrug-resistant *Salmonella* Typhimurium 4,[5],12:i:-DT193 strains carrying a novel genomic island adjacent to the *thrW* tRNA locus. *Int. J. Med. Microbiol.* **2010**, *300*, 279–288. [[CrossRef](#)] [[PubMed](#)]
6. Orsini, M.; Mangone, I.; DiPasquale, A.; Perticara, S.; Sacchini, L.; Cito, F.; Iannetti, S.; Marcacci, M.; Ancora, M.; Calistri, P.; *et al.* Draft genome sequences of 19 *Salmonella enterica* serovar Typhimurium [4,5:i:-] strains resistant to nalidixic acid from a long-term outbreak in Italy. *Genome Announc.* **2015**, *3*, 911–915.
7. Graziani, C.; Mughini-Gras, L.; Owczarek, S.; Dionisi, A.M.; Luzzi, I.; Busani, L. Distribution of *Salmonella enterica* isolates from human cases in Italy, 1980 to 2011. *Euro. Surveill* **2013**, *18*, 20519. [[CrossRef](#)] [[PubMed](#)]
8. Callaway, T.R.; Edrington, T.S.; Anderson, R.C.; Byrd, J.A.; Nisbet, D.J. Gastrointestinal microbial ecology and the safety of our food supply as related to *Salmonella*. *J. Anim. Sci.* **2008**, *86*, E163–E172. [[CrossRef](#)] [[PubMed](#)]

9. Lim, Y.H.; Hirose, K.; Izumiya, H.; Arakawa, E.; Takahashi, H.; Terajima, J.; Itoh, K.; Tamura, K.; Kim, S.I.; Watanabe, H. Multiplex polymerase chain reaction assay for selective detection of *Salmonella enterica* serovar Typhimurium. *Jpn. J. Infect. Dis.* **2003**, *56*, 151–155. [[PubMed](#)]
10. European Centre for Disease Prevention and Control. *Laboratory Standard Operating Procedure for MLVA of Salmonella enterica Serotype Typhimurium*; ECDC: Stockholm, Sweden, 2011.
11. Cito, F.; Baldinelli, F.; Calistri, P.; Di Giannatale, E.; Scavia, G.; Orsini, M.; Iannetti, S.; Sacchini, L.; Mangone, I.; Candeloro, L.; *et al.* Outbreak of unusual *Salmonella enterica* serovar Typhimurium monophasic variant 1,4 [5],12:i:-, Italy, June 2013 to September 2014. *Euro. Surveill* **2016**, *21*. [[CrossRef](#)] [[PubMed](#)]
12. Haeusle, G.M.; Curtis, N. Non-typhoidal *Salmonella* in children: Microbiology, epidemiology and treatment. *Adv. Exp. Med. Biol.* **2013**, *764*, 13–26.
13. Feasey, N.A.; Dougan, G.; Kingsley, R.A.; Heyderman, R.S.; Gordon, M.A. Invasive non-typhoidal salmonella disease: An emerging and neglected tropical disease in Africa. *Lancet* **2012**, *379*, 2489–2499. [[CrossRef](#)]
14. Hohmann, E.L. Nontyphoidal salmonellosis. *Clin. Infect. Dis.* **2001**, *32*, 263–269. [[PubMed](#)]
15. Pezone, I.; LaPenna, M.R.; Flamini, S.; Nigro, G. Non-typhoidal *Salmonella* septic arthritis in an immunocompetent child with a pharyngeal streptococcal infection. *Int. J. Infect. Dis.* **2009**, *13*, 35–36. [[CrossRef](#)] [[PubMed](#)]
16. Gordon, M.A.; Graham, S.M.; Walsh, A.L.; Wilson, L.; Phiri, A.; Molyneux, E.; Zijlstra, E.E.; Heyderman, R.S.; Hart, C.A.; Molyneux, M.E. Epidemics of invasive *Salmonella enterica* serovar Enteritidis and *S. enterica* serovar Typhimurium infection associated with multidrug resistance among adults and children in Malawi. *Clin. Infect. Dis.* **2008**, *46*, 963–969. [[CrossRef](#)] [[PubMed](#)]
17. Greene, S.K.; Daly, E.R.; Talbot, E.A.; Demma, L.J.; Holzbauer, S.; Patel, N.J.; Hill, T.A.; Walderhaug, M.O.; Hoekstra, R.M.; Lynch, M.F.; *et al.* Recurrent multistate outbreak of *Salmonella* Newport associated with tomatoes from contaminated fields, 2005. *Epidemiol. Infect.* **2008**, *136*, 157–165. [[CrossRef](#)] [[PubMed](#)]
18. US FDA and California Food Emergency Response Team. Investigation of the Taco John's *Escherichia coli* O157:H7 Outbreak Associated with Iceberg Lettuce. 2008. Available online: <http://www.cdph.ca.gov/pubsforms/Documents/fdb%20eru%20IceLet%20TacoJohn022008.pdf> (accessed on 29 April 2016).
19. Centers for Disease Control and Prevention. Outbreak of *Salmonella* serotype Saintpaul infections associated with multiple raw produce items—United States, 2008. *Morb. Mortal. Wkly. Rep.* **2008**, *57*, 929–934.
20. Fujimoto, T.; Tsuchiya, Y.; Shibuya, N.; Taiyoji, M.; Nishiwaki, T.; Nakamura, K.; Yamamoto, M. Contamination of the Shinano river water with mutagenic substances after the Niigata Chuetsu earthquake. *Tohoku J. Exp. Med.* **2007**, *211*, 171–180. [[CrossRef](#)] [[PubMed](#)]



© 2016 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC-BY) license (<http://creativecommons.org/licenses/by/4.0/>).