

Characterization of a foodborne outbreak caused by *Salmonella* Enteritidis in Aracaju, State of Sergipe, Brazil

Maria Regina Pires Carneiro^[1], Pedro Hernan Cabello^{[2],[3]},
Ricardo Luiz Cavalcanti Albuquerque-Junior^[4], Sona Jain^[5]
and Alexandre Luna Candido^[6]

[1]. Laboratório de Bacteriologia, Departamento de Morfologia, Universidade Federal de Sergipe, São Cristóvão, Sergipe, Brasil. [2]. Laboratório de Genética Humana, Escola de Ciências da Saúde, Universidade do Grande Rio, Rio de Janeiro, Brasil. [3]. Laboratório de Genética Humana, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, Brasil. [4]. Programa de Pós-Graduação em Saúde e Ambiente, Universidade Tiradentes, Aracaju, Sergipe, Brasil. [5]. Laboratório de Parasitologia, Departamento de Morfologia, Universidade Federal de Sergipe, Aracaju, Sergipe, Brasil. [6]. Laboratório de Virologia Comparada, Departamento de Morfologia, Universidade Federal de Sergipe, Aracaju, Sergipe, Brasil.

ABSTRACT

Introduction: In December 2001, an outbreak of foodborne gastroenteritis infected 114 of 161 people who ate at a restaurant in Aracaju, State of Sergipe, Brazil. **Methods:** The epidemiological and microbiological aspects of the outbreak were characterized. **Results:** Potato salad made with homemade mayonnaise and stored at unsuitable temperatures was associated with increased risk of foodborne infection. *Salmonella* Enteritidis was isolated from the diarrheal stools of the hospitalized patients, and genotyping of the fecal samples generated identical randomly amplified polymorphic deoxyribonucleic acid (DNA) profiles. **Conclusions:** To the best of our knowledge, this is the first and the only record of a gastrointestinal outbreak in Sergipe.

Keywords: Gastroenteritis. Poultry. *Salmonella* Enteritidis.

Foodborne diarrheal outbreaks represent an important global health problem⁽¹⁾. Recent studies estimate that there are 80.3 million annual cases of *Salmonella*-related diseases worldwide⁽²⁾. *Salmonella* is an enteroinvasive pathogen that most commonly causes self-limiting gastroenteritis. Approximately 5% of all patients develop septicemia, and the effect on children, elderly, and immunocompromised patients can lead to more serious complications, including death^(3,4).

Salmonella Enteritidis is considered the most common serovar in human infections⁽⁵⁾, and most of these infections are associated with poultry products⁽⁶⁾. Since 1993, Enteritidis has also been the most frequent serotype responsible for foodborne outbreaks and sporadic cases of gastrointestinal and septicemic diseases in Brazil^(7,8).

There were no recorded cases in Sergipe before 2001, when a gastroenteric outbreak occurred among those who consumed food prepared in a restaurant in the City of Aracaju (latitude 10°54'15"; longitude 37°02'40"), Sergipe, Brazil, on December 2, 2001. The outbreak was recorded via an

epidemiological enquiry that was conducted by the *Serviço de Vigilância Epidemiológica da Secretaria Municipal de Saúde* (SVE-SMS) of Aracaju, which is the Epidemiological Service of the Health Department of the municipality of Aracaju. The main objective of the present study was to describe the epidemiological and microbiological characteristics of this foodborne outbreak of *S.* Enteritidis caused by AJU-SE021201 clone MR05.

A cohort study was conducted, in which all sick individuals (patients) and individuals who were not sick (controls) who consumed the food prepared on December 2, 2001 in the restaurant in Aracaju were retrospectively interviewed few days after the outbreak. A questionnaire following the standard form of the SVE-SMS of Aracaju for outbreak investigations was used to record the food(s) consumed; signs or symptoms such as diarrhea, vomiting, nausea, fever, cephalalgia, discomfort, or abdominal pain during the 5 to 86 hours after food consumption; time of symptom appearance; incubation period; and relationship between exposure and illness. The median incubation period was calculated. For each food item consumed, the attack rate (AR) and relative risk (RR) with the corresponding 95% confidence interval (CI) and *p* value were calculated using Statistical Package for the Social Sciences (SPSS), v12.0 (SPSS Corp, Chicago, IL, USA)⁽⁹⁾ and Epi-Info version 6.4 (Centers for Disease Control and Prevention, Atlanta, GA, USA)⁽¹⁰⁾.

The sanitary inspection was carried out by SVE/SMS agents of Aracaju City who examined the sanitary-hygienic, and physical conditions, as well as procedures to trace the

Corresponding author: Dra. Maria Regina Pires Carneiro. Laboratório de Bacteriologia/Depto de Morfologia/UFS. Av. Marechal Rondon s/n, Cidade Universitária Prof. José Aloísio de Campos, Jardim Rosa Elze, 49100-000 São Cristóvão, Sergipe, Brasil.

Phone: 55 79 8128-6970

e-mail: profregina@hotmail.com

Received 27 October 2014

Accepted 24 February 2015

commercialized foods, and identify the risk factors that contributed to the outbreak. Coproculture exams were conducted with 10 diarrheal stool samples obtained from the hospitalized patients who had consumed the food in the restaurant in question. Isolated samples were biotyped using the Enterobacterias ID 32 E (bioMérieux, Craponne France) identification system and antibiographed using the disk diffusion method in the *Laboratório de Virologia Comparada (LVC), Departamento de Morfologia (DMO)* of the *Federal University of Sergipe (UFS)*. The following antimicrobials were tested: ampicillin (10µg), chloramphenicol (30µg), nitrofurantoin (300µg), norfloxacin (10µg), streptomycin (10µg), tetracycline (30µg), and trimethoprim-sulfamethoxazole (30µg). For serotyping and phagotyping, the samples were sent to the *Laboratório de Enteropatógenos* of the Department of Bacteriology of *Fundação Oswaldo Cruz (FIOCRUZ)*; Rio de Janeiro. To confirm the identity of the various isolated coprocultures, the samples were subjected to molecular typing using randomly amplified polymorphic deoxyribonucleic acid (DNA) restriction fragment length polymorphism-polymerase chain reaction (RAPD-PCR). The Ready-To-Go RAPD-PCR Analysis Beads system (Amersham Biosciences, Piscataway, USA) was used, including the supplied primers 1 (5'-GGTGC GGAA-3') and 4 (5'-AAGAGCCCGT-3'). The initial denaturation was carried out at 95°C in a thermo-cycler (Thermo Hybrid PCR Sprint, Thermo Scientific, Waltham, USA) for 5 minutes, followed by 40 cycles at 94°C for 30 seconds, 35°C for 1 minute, and 72°C for 2 minutes. A final run at 72°C for 5 minutes was also carried out. The amplified products were electrophoresed using 5% polyacrylamide gel and visualized after coloring with silver nitrate using PlusOne DNA Silver Staining Kit™ (Amersham Biosciences).

Pathogenicity tests were carried out after approval by the Animal Bioethics Committee of the Federal University of Sergipe under the reference number 16/04. The strain identified as *S. Enteritidis* sample AJU-SE021201 clone MR05 was

inoculated, orally into male Swiss mice (*Mus musculus*), aged between six to nine weeks, acquired from the vivarium of the Federal University of Sergipe. Clinical signs were monitored for 7 days after the inoculation, by observation and weighing. After being sacrificed in a humanitarian manner, leucograms and histopathological exams of the liver, spleen, kidneys, small and large intestines and lungs were carried out.

Of 161 individuals interviewed, 114 (70.8%) had gastroenteric symptoms. The median incubation period was 13 hours and ranged from 1 to 30 hours, which agrees with well-known incubation periods for salmonellosis cases⁽⁴⁾.

The relationship between the commercialized foods and the number of persons who were exposed (people who consumed the food) or not exposed (people who did not consume the food) to each of the foodstuffs and the patients or controls are presented in **Table 1**. The association between the potato salad with mayonnaise and the disease was significant (RR= 2.12; 95% CI: 1.41-3.17; p < 0.0001). The statistical significance of chicken and macaroni (**Table 1**) does not mean that there was more than one transmission vehicle. Macaroni was mainly an accompanying dish and it is possible that there was contamination via fomites. Some people who did not eat the potato salad with mayonnaise became sick due to cross-contamination from utensils or equipment.

The inquiry conducted at the establishment revealed that the food remained at an ambient temperature for approximately 18 hours, a critical point of control in the preparation of foodstuffs as per hazard analysis critical control point (HACCP)⁽¹¹⁾.

Presence of *S. Enteritidis* was confirmed in all of the diarrheal stool cultures. These samples were non-phagotypes, sensitive to all of the antimicrobials tested except nitrofurantoin (possibly due to indiscriminate use of this antibiotic or products containing this antibiotic for disinfection, hygiene, or treatment in poultry houses), and produced identical genotypic profiles

TABLE 1 - Risk analysis of the food associated with the outbreak of gastroenteritis by *Salmonella* Enteritidis strain AJU-SE021201.

Food consumed	Exposed (e)				Not exposed (o)				RR	95% CI	P value
	D _e	S _e	total	AR _e	D _o	S _o	total	AR _o			
Potato salad with mayonnaise	71	8	79	89.87	14	19	33	42.40	2.12	1.41–3.17	<0.0001
Chicken	57	9	66	86.36	28	18	46	60.87	1.42	1.10–1.82	0.0025
Rice	40	16	56	71.43	45	11	56	80.36	0.89	0.72–1.10	0.2711
Herdman beans	53	16	69	76.90	32	11	43	74.40	1.03	0.83–1.28	0.7530
Manioc flour	2	6	8	25.00	83	21	104	79.80	0.31	0.09–1.04	0.0015
Fish	1	1	2	50.00	84	26	110	76.36	0.65	0.16–2.63	0.3877
Macaroni	4	7	11	36.36	81	20	101	80.20	0.48	0.21–0.99	0.0023
Chips	9	1	10	90.00	76	26	102	74.51	1.21	0.95–1.53	0.3297
Green vegetable salad	5	6	11	45.45	80	21	101	79.21	0.57	0.30–1.10	0.0141
Vinegar source	9	1	3	33.33	84	25	109	77.06	1.17	0.93–1.47	0.4219

D: sick; S: healthy; AR: attack rate; RR: relative risk; CI: confidence interval.

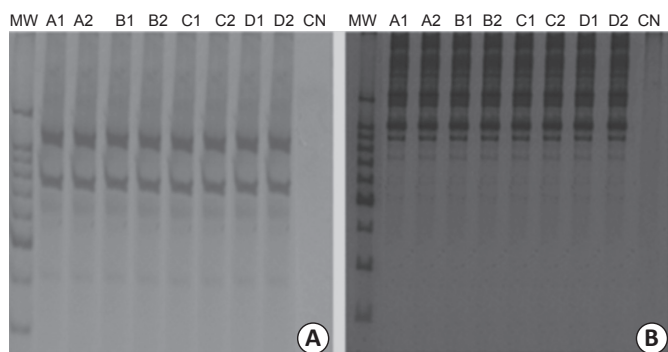


FIGURE 1 - Randomly amplified polymorphic DNA (RAPD) profile of four isolates from diarrheal stool samples, each from two different coprocultures using primer 1 (Figure 1A) and primer 4 (Figure 1B). Lane 1 100bp DNA ladder; MW (molecular weight); A1, B1, C1, D1 (coproculture 1); A2, B2, C2, D2: coproculture 2; NC (negative control). DNA: deoxyribonucleic acid; RAPD: restriction fragment length polymorphism.

using both primers with RAPD-PCR (Figure 1A and Figure 1B). The strain in question was catalogued as *S. Enteritidis* AJU-SE021201 clone MR05. Oral inoculation of this strain in Swiss mice (*Mus musculus*) resulted in a delay in body growth, and histopathological exams of the liver, spleen, kidneys, small and large intestines, and lungs (results not shown) demonstrated that the sample responsible for the outbreak provoked symptoms of foodborne intoxication.

Epidemiological evidence suggested a strong association between the consumption of potato salad with mayonnaise and the infection. Microbiological analysis of the commercialized foods, preparation area, and food handlers would be relevant to identify the presence of *S. Enteritidis* with identical characteristics as those isolated from the stool samples of the patients. However, as is often the case, those responsible for the commercial establishment implemented vigilant interventions to avoid heavy fines and eventual closure. The food was rapidly discarded and the utensils sterilized, making it difficult to determine the route of the infection. Importantly, all of the people affected in this outbreak had a similar history; they all ingested the same food prepared in the same restaurant and on the same day and at the same time.

It was confirmed that the mayonnaise was made at the restaurant and left at an ambient temperature, which was around 30°C on the day of the outbreak, that favored multiplication of the bacteria. Raw eggs used for the mayonnaise are the probable source of the *S. Enteritidis*.

To reduce foodborne illnesses, it is important to publicize information concerning the vehicles and causes of food poisoning, and individualization of pathogenic samples is essential to study the association between clinical cases and the possible sources of infection. Since 2001, there has been no report of a gastroenteritis outbreak in Sergipe. The few reports available on Enteritidis outbreaks in Brazil are from the Southern states of Brazil⁽¹²⁾⁽¹³⁾. However, reduced numbers do not always represent a real decrease in the number of outbreaks, but instead could be representative of under reporting of the outbreaks.

As most of the gastroenteritis cases resolve without the need for hospitalization and without isolation of the causative agent in the incriminated food, the occurrence of salmonellosis transmitted by food in the human population is probably underestimated. Forsythe⁽¹⁴⁾ stated that only 10% of all foodborne outbreaks are reported in Brazil, due to flaws in the reporting and monitoring system. According to the Brazilian Health Ministry⁽¹⁵⁾, 6,062 cases of foodborne outbreaks were reported in Brazil from 1998-2008. In 51% of these cases, it was not possible to establish the etiologic agent, and in 34.3% of the cases, the source of infection was not identified. These data demonstrate the absence of an adequate information system capable of tracking and identifying outbreaks across the country.

The present study contributes to the characterization of the first and only foodborne outbreak recorded in the City of Aracaju, State of Sergipe and demonstrates an urgent need for a better reporting and monitoring system as well as better food safety procedures that are important for the control of foodborne infectious diseases. The use of good practices in the production and conservation of food are essential to ensure that food is fit for consumption.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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