

Salmonella Prevalence in Fresh Cheese and its Incidence in Public Health

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Abstract

Fundamentals: Salmonellosis is a bacterial infectious disease and dangerous to humans. It is caused by Salmonella Typhi and paratyphi resulted from eating contaminated food. The objective of this study was to demonstrate the prevalence of Salmonella in fresh artisanal cheeses.

Methods: 25 grams of each sample were weighed and homogenized in 225 ml of simple lactosate broth which was incubated for 24 h at 37°C. Then 1 ml of pre-enrichment culture was transferred in selenite-cystine broth and 1 ml in tetrathionate broth for selective enrichment. Both tubes were incubated at 37°C/24hours. From these, the culture was streaked on Salmonella - Shigella agar and bismuth sulfite agar and incubated both in inverted position at 37°C/24hours.

Results: The cheese microbiological analysis reported values ranging from absence to 280666.67 CFU/g of Salmonella. The results obtained showed that the presence of Salmonella was 80% prevalent in the fresh artisanal cheeses analyzed which are sold in the public markets of Riobamba City.

Conclusions: Eight out of the ten brands analyzed showed the presence of Salmonella showing the unsafe practices during the artisanal cheese manufacture, transportation without cold chain and marketability without refrigeration in four public markets.

Keywords: *Salmonellosis, bacterial infectious, Cheese.*

INTRODUCTION

Salmonella is a Gram-negative, spontaneous anaerobic microorganism of the Enterobacteriaceae family. It is characterized by its mobility and its lack of ability to produce lactose fermentation. It is able to do so gradually with the production of acid and gas from glucose. This differs from Salmonella enterica serotype Typhi which does not have the ability to generate gas; however, it stands out for its ability to produce hydrogen sulfide (1)

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Over 2500 *Salmonella* serotypes have been identified: among them, *Salmonella* Enteritidis and *Salmonella* Typhimurium are the most frequently isolated serotypes causing human disease worldwide (2).

The pathogenic bacteria, among which, the *Salmonella* genus is identified cause a wide spectrum of diseases ranging from the asymptomatic carrier to typhoid fever, which clinical expression is more severe, to gastroenteritis and enterocolitis which are usually more frequent manifestations. When infection is caused by the types known as typhoid, especially Typhi and Paratyphi, it manifests itself as a systemic disease that could be lethal, which is why it is called enteric fever or typhoid fever and presents high fever symptoms ($> 38^{\circ}\text{C}$), nausea and headache. Moreover, it could produce neurological affectations, intestinal perforation and death. When infection is caused by non-typhoidal serotypes, the disease symptoms usually begin 12-72 hours after infection and causes diarrhea linked to fever and abdominal pain. (3) In most cases, in healthy individuals, infection is self-limited and resolves in 4-7 days. In some people, these non-typhoidal serotypes manage to spread to other areas of the organism and give rise to difficulties such as endocarditis, osteomyelitis, etc. (3).

The pathogenic bacterium *Salmonella* continuously causes foodborne illnesses, better known as "food poisoning". The CDC estimates that *Salmonella* causes 1 million cases of foodborne illness in the United States each year (4). In recent years, outbreaks of salmonellosis have triggered 1 million people to become ill annually in the United States, generating high health care costs, (5) (U.S. National Library of Medicine, 2018).

Food from animal origin remains the main source of TADs worldwide, such as Norovirus, *Salmonella* enterica non-typhoid and *Campylobacter* spp. It is estimated that *Salmonella* notifoidea causes 80 million cases of infection and 60,000 deaths annually while *Campylobacter* spp. causes 95 million cases of infection and 21,000 deaths (6).

Salmonella outbreaks (7) are a direct consequence of consumption of contaminated eggs, poultry and mammalian meats, milk, fruits and vegetables; in addition, some companion animals (e.g., turtle, dog, cats) become vectors of *Salmonella* (WHO, 2018).

Among foodborne illnesses, non-typhoidal *Salmonella* (in reference to salmonellosis) is one of the three more important pathogens which promote foodborne illness (8). Salmonellosis has long been associated with animal products, but recently there has been an increase in the incidence of fresh produce.

In 2018, EU member states reported 5,146 foodborne outbreaks affecting 48,365 people. Salmonellosis was the second most frequently reported gastrointestinal infection after campylobacteriosis (9).

Salmonellosis is the second most common zoonotic disease after campylobacteriosis in the European Union (EU), and *Salmonella* is a common cause of foodborne disease outbreaks.

More than 91,000 cases of salmonellosis are reported each year in the EU. EFSA has estimated that the overall economic burden of human salmonellosis could account for 3.000 million euros per year. After several years of decline, salmonellosis cases in the EU have leveled off. In 2018, Member States reported 91 857 cases (9).

EU-wide statistics on human salmonellosis and *Salmonella* envelopes in food and animals, respectively, during 2014-2018. They report a total of 198.552 cases of salmonellosis from consumption of milk and dairy products in 24 EU member states (10).

In Ecuador (11) during 2019, water and foodborne diseases reached around 19.500 cases showing a decrease compared to 2018 with 24.000 cases.

198 cases of typhoid and paratyphoid fever have been reported up to week 18 of 2021. From the total number of cases nationwide, the province with the highest number of cases is Esmeraldas with 51 cases. The most affected age group is 20 to 49 years old (12).

The Ecuadorian Institute for Standardization, a national public sector entity of a scientific and technological nature, defines fresh cheese as: unripened, not scalded, molded, slightly firm and granulated, and may be made with whole milk, semi-skimmed, coagulated by enzymatic means or using organic acids, without the addition of lactic cultures: in addition, the same technical standard NTE INEN 1529-7 specifies the microbiological requirements for fresh cheeses, establishing the absence of *Salmonella* in a sample of 25 g.(13)

In the jurisdiction of Riobamba Canton, the presence of *Salmonella* spp in three rural cheese factories (14) reported values ranging from 301962.96 CFU/g to 989777.78 CFU/g, which confirms the incidence of *Salmonella* in foods from animal origin, as is the case of fresh cheese.

Albuja (2018) evaluated the quality of fresh cheeses in three rural cheese factories, which are not suitable for human consumption, because they do not meet the microbiological criteria of INEN Standard 1529-7 (15).

In another study (16) conducted in Chone Canton, the presence of salmonella in 28 rural cheese factories out of 35 analyzed was evidenced. It was established that 80% of producers do not comply with hygiene standards and microbiological requirements of NTE INEN 1528 (2012) due to the poor conditions in the process and quality of milk to produce fresh cheese.

The presence of *Salmonella* spp. was evaluated in fresh cheeses sold in the grocery stores of Simon Bolivar Canton and contrasted with the amount of cheese purchased for sale in 25 stores analyzed in the first sampling, 11 stores (44%) had *Salmonella* spp. and 56% (14) stores did not report this bacterium. In a second sampling, 60% (15) stores were positive for *Salmonella* spp. and 40% (10) stores obtained negative results (17).

METHODS

For this study, a random sampling was applied to 90 samples of fresh artisanal cheeses packed in polyethylene bags of different brands sold in public markets in the city of Riobamba, acquired on different days and at different times; the circumstances of handling and conservation methods were equivalent.

Twenty-five grams of each sample were weighed and homogenized in 225 ml of simple lactosate broth which was incubated for 24 h at 37°C. Then 1 ml of pre-enrichment culture was transferred in selenite-cystine broth and 1 ml in tetrathionate broth for selective enrichment, both tubes were incubated at 37°C/24 hours. From these, the culture was streaked on *Salmonella* - *Shigella* agar and on bismuth sulfite agar and incubated both in inverted position at 37°C/24 hours (18).

Treatment and experimental design.

The experimental units in the present investigation were 90 samples of fresh cheese; sampled monthly, transferred in thermal boxes for a period of one hour to the laboratory for microbiological analysis using the method.

One gram of each cheese sample was weighed. Distilled water was added to three test tubes. The previously weighed cheese sample was added to one of the tubes and shaken for three minutes, then dilutions were made up to 103, and from this dilution, 1 ml was placed in the plates to determine the presence or absence of *Salmonella*. The plates were incubated at 37 °C for 24 hours, and then counted.

Statistical analysis

The results of the laboratory analysis of the fresh artisanal cheese samples were calculated with Infostat 2020 ® software to determine the level of contamination with *Salmonella* spp and the separation of means at the significance level of ($p > 0.05$).

RESULTS

Sampling of fresh artisanal cheeses was carried out in four municipal markets in the urban area of Riobamba city based on the quantity of cheeses and different brands sold at the counters of the markets: La Condamine, San Alfonso, Santa Rosa and Mayorista. This study focused mainly on determining the presence or absence of *Salmonella*. 48 samples were positive for *Salmonella* and 12 samples were absent, which confirmed the presence of *Salmonella* in 80% of the analyzed cheeses. According to the results of this research work at the laboratory level, the lack of safety of fresh artisanal cheeses distributed in four municipal markets in the canton of Riobamba and the level of risk that the presence of *Salmonella* means for the health of consumers was confirmed. The presence of pathogenic microorganisms in food is one of the problems of public health interest.

The microbiological analyses reported the presence of *Salmonella*. Significant differences were observed between the mean values of the samples of fresh artisanal cheeses analyzed at the laboratory level (Table 1), showing that 80% of the samples of fresh cheeses of the 10 different brands analyzed were contaminated with this pathogenic bacterium, where its quantification ranges from 0.0 CFU/g (absence) to 280666.67 CFU/g.

Table 1. Prevalence of *Salmonella* in fresh artisanal cheeses marketed in the city of Riobamba, expressed in CFU/g.

Cheeses	Measures	N	E.E.		Prob.
QFA1	280666,67	6	2756,50	E	0,0001
QFA8	69166,67	6	2756,50	D	0,0001
QFA6	44600,00	6	2756,50	C	0,0001
QFA7	19766,67	6	2756,50	B	0,0001
QFA4	6646,67	6	2756,50	A	0,0001
QFA5	5508,33	6	2756,50	A	0,0001
QFA2	4072,67	6	2756,50	A	0,0001
QFA3	3,17	6	2756,50	A	0,0001
QFA9	Absence	6	2756,50	A	0,0001
QFA10	Absence	6	2756,50	A	0,0001

Means with a common letter are not significantly different ($p > 0.05$).

The lowest values of the prevalence of *Salmonella* are found in the samples of fresh artisanal cheeses with the codes, while the cheeses with QFA9 and QFA10 with absence (0.0)CFU/g. and QFA3 with 3,17 CFU/g, while codes QFA1 and QFA8, presented the highest values with 69166,67 CFU/g and 280666,67CFU/g respectively, values that do not comply with NTE INEN 1528:202, which makes them unfit for human consumption and of high risk to trigger salmonellosis problems in consumers.

DISCUSSION

Cheeses with values between 103 and 105 (10000.00 to 100000.00 CFU/g) represent a high risk to health by consumption of fresh cheeses, the values fluctuate from 3,17 to 280666,67 CFU/, whose infectious dose with viable bacteria capable of colonizing the intestine according to some authors that with only 10 bacteria can cause gastroenteritis in the most susceptible groups to infection (19).

Fresh artisanal cheeses with microbiological loads of 105 (100000.00 CFU/g) constitute a potentially dangerous threat for the healthy population who acquires and consumes this type of soft cheeses, due to the fact that typhoid salmonellosis having specifically human reservoirs, according to studies with healthy volunteers have presented that an inoculum as small as 105 organisms of *Salmonella Typhi* causes infection in more than 50% of healthy volunteers (20); however, other investigators mention the infectious dose in 103 bacteria. *Salmonella. Paratyphi* requires a larger inoculum to cause infection.

Non-typhoidal salmonellosis is an important zoonosis, because domestic and wild animals are carriers of *Salmonella*, including poultry, reptiles, cattle, rodents, domestic animals, birds and humans, and meat products, especially poultry, eggs and dairy products such as fresh cheeses, are the most common vehicles of salmonellosis (20, 21).

In recent years, fresh produce such as fruits and vegetables have become of interest as vehicles of transmission. *Salmonella* can adhere to food contact surfaces and cause cross-contamination, unhygienically handled food and even more so from individuals carrying *Salmonella. Typhi* or vegetables contaminated by irrigation with sewage. Because of its low infective dose, it can also be transmitted from individual to individual (21).

Based on the results of the present study with 10 brands of fresh cheeses analyzed from the four municipal markets of the city of Riobamba, it can be mentioned that the prevalence of salmonella is high and therefore these types of contaminated cheeses are potentially likely to cause foodborne illnesses, specifically salmonellosis. This is related to other studies developed in Ecuador, recently in the canton of Riobamba, it was investigated that fresh cheeses produced in rural cheese factories determined the presence of *Salmonella* in 100% of the cheeses analyzed with values of 997555.56 CFU/g, 219111.11 CFU/g and 22222.22 CFU/g in different cheese factories with an average storage temperature of 12 °C. The results showed to be the consequence of places with little care for safety in their production (14). There is also another study carried out in the city of Guayaquil at the supermarket level where fresh cheeses marketed and refrigerated were analyzed, determining that 16% of the samples were positive for the presence of *Salmonella* in fresh cheeses kept refrigerated at the supermarket level (22).

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