

OCCURRENCE OF *SALMONELLA* SP. AND COAGULASE-POSITIVE STAPHYLOCOCCI IN RAW EGGS AND COALHO CHEESE: COMPARATIVE STUDY BETWEEN TWO CITIES OF BRAZIL'S NORTHEAST

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ABSTRACT

Microbiological analyses of chicken eggs in Recife and Salvador have shown a high occurrence of *Salmonella* in the egg shells and yolks. Likewise, the occurrence of *Salmonella* plus coagulase-positive staphylococci in Coalho cheese reached alarming levels. The data revealed a significant risk of infections and intoxications from consuming these foods in the cities.

Key words: *Salmonella*, *Staphylococcus*, Coalho cheese, eggs.

Risk of foodborne outbreaks in developing countries is usually reported as derived from unsanitary conditions, improper food storage and lack of hygiene during preparation of food products (7). Although Brazilian law related to food protection and surveillance is in accordance to international rules, epidemiological studies regarding foodborne pathogens involved in Brazilian outbreaks are rare. Especial attention has been paid for raw or undercooked eggs because the hens act as natural reservoirs of *Salmonella* sp. strains causing gastroenteritis. In United States there are 1.4 million cases of salmonellosis yearly and commercially produced eggs have caused major epidemics due to *Salmonella enterica* serotype Enteritidis (4). In Brazil, outbreaks of salmonellosis apparently occurred because of consumption of contaminated mayonnaise or sweet creams (14), which are commonly prepared with eggs. The contamination occurs through the shell (15); but humidity,

temperature and storage time are critical for migration of bacteria from the surface of the shell to the inner structures of the egg.

Also, farmhouse cheese is typically consumed in population's daily life in many parts of the globe. However, the occurrence of *Salmonella* sp. besides coagulase-positive staphylococci in Coalho cheese, a typical product of Brazil's northeast region, has been recorded (3, 8). The cheese is generally produced in small operations, mostly made from raw milk with the rennet obtained from cow stomachs due to the presence of the enzyme renin (9). After food contamination, staphylococci are able to release thermostable enterotoxins that remain active in food and are resistant to proteolytic enzymes of the human intestinal tract (2, 5). Therefore, continuous surveillance is necessary to create helpful strategies to prevent food poisoning and staphylococcal intoxications.

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The aim of the present study was to record the level of contamination of raw eggs by *Salmonella* sp., besides the occurrence of *Salmonella* sp. plus coagulase-positive staphylococci in Coalho cheese in two major cities of Brazil's northeast, Salvador and Recife. Thus, the risk for human health from consuming these foods are discussed in light of previous studies carried out in the region.

The food analyses were carried out following the recommendations of Downes and Ito (7) at the Laboratory of Food Analyses (UNIME/ Salvador). Raw eggs and Coalho cheese were purchased at stores of nine small markets of Recife and Salvador, where no information about the food origin or expiration date was informed. Samples of three yolks or egg shells were evaluated as a pool whereas individual pieces of cheese were weighted, and 25 grams were diluted in 225 mL (w:v) of 1% peptone water and incubated for 24h at 37 °C. For *Salmonella* analyses, 1 mL was inoculated into Selenite-Cystine or Tetrionate-Novobiocin broth for 24h at 37 °C. After this time, the samples were cultured in Salmonella-Shigella agar, xylose-lysine-deoxycholate agar or MacConkey agar and typical colonies were sub-cultured in tubes containing triple-sugar-iron agar. Then, suspect isolates were submitted to biochemical tests to determine the genus.

The search for coagulase-positive staphylococci was carried out after culturing samples in Baird-Parker agar for 24-48h at 37 °C. The colony forming units were enumerated (CFU/ g), and three typical colonies were selected for the

coagulase, catalase and Gram-staining tests. Samples contaminated by coagulase-positive staphylococci were considered not proper for human consuming when the number of colony forming units reached above 10^3 CFU/ g. Results were expressed as percentage of positive samples. Data were analyzed statistically by the Student *t*-test or ANOVA, with the level of significance set at $p < 0.05$.

Occurrence of *Salmonella* sp. in egg shells and yolks ranged from 11.25 - 25 % in the cities of Recife and Salvador (Table 1). Although the pathogen was more frequently detected in yolks than in egg shells in samples from Salvador, the overall contamination was similar between the two cities ($p > 0.05$). Among outbreaks of salmonellosis recorded from July 1993 to June 1997, in São Paulo State, 95.7% were related to consumption of food containing raw or undercooked eggs (10). The present data reinforced that contaminated eggs are common sources of infection through food products, and increases the chance of consumers to become ill. While Brazilian Law (11) established the lack of *Salmonella* sp. in food products, these regulations are not uniformly enforced due to unofficial commerce of poultry products. In addition, eggs sold at markets of Salvador and Recife were often maintained at ambient temperature, which ranges from 27 - 35 °C during the whole year in Brazil's northeast. This temperature range is ideal for proliferation of mesophyly bacteria, such as *Salmonella* strains.

Table 1. Occurrence of *Salmonella* sp. in chicken eggs in Salvador and Recife, Brazil's northeast.

	Markets										
	Salvador					Recife					Total in Salvador
	A	B	C	D	E	A	B	C	D		
Number of eggs	36	84	36	48	36	60	36	36	48	240	180
Pool of samples	12	28	12	16	12	20	12	12	16	80	60
Number of positive pools of shells	01	02	01	02	03	04	03	04	04	09	15
Shells (%)	8.33	7.14	8.33	12.5	25	20	25	33.33	25	11.25	25
Number of positive pools of yolks	02	03	01	03	04	04	03	04	04	13	15
Yolks (%)	16.66	10.72	8.33	18.75	33.33	20	25	33.33	25	16.25	25

Food outbreaks related to staphylococcal strains were previously recorded with 180 people in São Paulo (6). Also, the incidence of vancomycin-resistant staphylococci reached 90.9 % in Coalho cheese obtained in different states of Brazil's northeast (12). In the present study, the contamination levels of the cheese by *Salmonella* sp. ranged from 12.7 - 7.8 % and coagulase-positive staphylococci ranged 21-31.2% in Salvador and Recife, respectively (Table 2). Due to high occurrence of *Salmonella* and coagulase-positive staphylococci above the legal threshold of 10³ CFU/ g, samples were not proper for human consuming (Table 2). Previous studies carried out in other states of northeast region have

shown contamination levels by *Salmonella* ranging from 9 - 34.9 %; and by coagulase-positive staphylococci ranging from 23.3 - 72.7 %, respectively (1, 3, 8, 9, 13). Nevertheless, a significant amount of samples were concurrently contaminated by *Salmonella* and staphylococci (Table 2). Comparatively, the occurrence of *Salmonella* sp. in cheese samples from Salvador and Recife was considered low; but the occurrence of staphylococci was high and more prevalent in samples from Recife than Salvador ($p < 0.05$). Taken together, it is clear that risk of food poisoning outbreaks from consuming Coalho cheese is spreading in all states of the region.

Table 2. Percentage of samples of Coalho cheese not proper for human consuming in Salvador and Recife.

Markets in Salvador	Number of samples	<i>Sal</i> ^a (%)	<i>Staph</i> ^b (%)	<i>Sal</i> + <i>Staph</i> ^c (%)
A	15	20	46.6	13.3
B	15	26.6	40	20
C	15	20	26.6	6.6
D	15	33.3	40	13.3
E	15	13.3	33.3	13.3
N = 75 % out of 75:		12.7	21	7.5
Markets in Recife	Number of samples	<i>Sal</i> ^a (%)	<i>Staph</i> ^b (%)	<i>Sal</i> + <i>Staph</i> ^c (%)
A	18	22.2	88.8	16.6
B	16	18.7	62.5	18.7
C	15	20	80	20
D	16	12.5	62.5	6.2
N = 65 % out of 65:		7.8	31.2	6.5

a. *Sal*: *Salmonella* sp.

b. *Staph*: Coagulase-positive staphylococci - only samples with colony forming units higher than 10³ CFU/g were considered not proper for human consuming (ANVISA, RDC 12, 02/01/2001).

c. Percentage of samples concurrently contaminated with *Salmonella* sp. and coagulase-positive staphylococci.

The present data indicates an emergent risk for human public health due to consuming of Coalho cheese and raw or undercooked eggs in Recife and Salvador, two major cities of Brazil's northeast. As a practical application, the data reinforced that continuous food surveillance is necessary to minimize the chance of food poisoning and infection outbreaks in the region.

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