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Hygienic and Sanitary Conditions of Eggs Commercialized in Town Fairs and Markets

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Abstract

High nutritional content coupled with low cost and ease of purchase justifies the frequent use of chicken eggs. Even with technological innovations in the production, storage, and distribution of eggs, food poisoning continues to occur. Therefore, in the present study, we 1) evaluated the microbiological profile and the hygienic and sanitary quality of eggs commercialized in town fairs and markets and 2) verified whether there is any correlation between microbiological results and commercialization conditions. Thirty-six samples were collected from six municipalities in the region of Recôncavo from Bahia, Brazil. The hygienic and sanitary conditions were analyzed by quantifying the total coliforms, *E. coli*, mesophilic and psychrotrophic microorganisms, molds, and yeasts, and by the presence of *Salmonella* sp. The observational analysis showed that 91% and 68% of the samples in town fairs and markets, respectively, did not comply with the requirements of the current Brazilian legislation. There was no significant difference in the microbiological profile among samples from the fairs and markets. Several samples presented relevant significant values, indicating inadequate hygienic conditions and fecal contamination. *Salmonella* sp. was found in one sample. There was co-relationship between microorganism count and the variables "external dirt", "external stains", and "closeness to other products", with high concentrations of total coliforms and *E. coli* in the non-conforming samples. Although no limits exist for mold and yeast counts, the presence of fungi makes the product unfit for consumption. Thus, the commercialization of eggs would benefit from more attention and supervision by the relevant authorities.

Keywords

Contamination, Egg, *Salmonella*, Microorganism Indicator, Hygiene Condition

1. Introduction

Eggs are perhaps the most nutritious food in nature, as they represent a low-cost source of high protein contents. However, worldwide epidemiological data have shown that they may also be a source of *Salmonella*, causing infections in human [1] [2] [3]. In their review on food contamination by *Salmonella* in several countries, Carrasco *et al.* [4] reported that the food of animal origin are the main source of contamination by *Salmonella*; among these, eggs and egg-based products are conspicuous.

Considering the foods that caused disease outbreaks in Brazil between 2007 and 2016, eggs and egg-based products ranked third, behind only to mixed food and water [5]. Most food contamination outbreaks in Brazil are due to bacteria of the genus *Salmonella*, an important zoonosis agent, posing public health challenge due to its high morbidity and the difficulty associated with its control [5] [6] [7]. Brazilian legislation stipulates the absence of *Salmonella* spp. in 25 g of eggs, that is, it mandates the absence of *Salmonella* spp. in a 25 g product for it to be considered safe for consumption [8]. Besides *Salmonella* spp., the presence of other organisms, such as mesophilic aerobic bacteria, psychrotrophic microorganisms, and total and thermotolerant coliforms, is also an important sign that the food (eggs) is unhealthy, indicating microbiologic contamination and lack of hygienic and sanitary conditions of food [9].

Pathogens recovered from eggs in the past included the following species: *Aeromonas*, *Campylobacter*, *Listeria*, and *Salmonella*. However, in terms of reported outbreaks of illness attributed to eggs, only *Salmonella* is of significance.

Considering that there are only a few studies in Brazil on pathogenic microorganisms and microbiological characterization in eggs, the objective of the present study was to evaluate the microbiological profile of eggs commercialized in public fairs and markets in the municipalities of Recôncavo from Bahia region, Brazil.

2. Materials and Methods

2.1. Sample Collection

The present analysis was carried out between December 2015 and January 2016, and encompassed six municipalities in the Recôncavo da Bahia Region, Brazil: Nazaré, Santo Antônio de Jesus, Cachoeira, Santo Amaro, Cruz das Almas, and São Felipe (39˚06'26"S latitude and 12˚40'39"W longitude, with an altitude of 226 m). Three market suppliers and three town fair suppliers from the above mentioned towns were selected for egg collection. Samples were purchased and each sample unit comprised six eggs per selling firm; a total of 36 samples from 18 markets and 18 fairs.

2.2. Analysis of Hygienic and Sanitary Conditions

An observational analysis was performed upon the retrieval of egg samples. This employed a verification list based on Decrees RDC No. 275/2002, RDC No. 216/2004, and RDC No. 35/2009, defining hygienic and sanitary conditions for

the commercialization site, eggs, and handlers. These laws emphasize the use of the guidelines that compose the standards of good food manufacturing practices, such as standards of good practices for food services, as well as mandatory maintenance and consumption instructions for egg labeling [10] [11] [12].

The percent of commercial sites complying or not complying with the above Decrees was identified based on the data obtained from the verification list. A correlation study between the results of observational and microbiological analyses was performed.

2.3. Sample Preparation

The samples were prepared as described by Silva *et al.* [9], with some modifications. Each egg was washed with water and neutral detergent, rinsed, and immersed in 70% alcohol for 10 min. The eggs were broken aseptically using sterile gloves, and the content was placed in a bowl and homogenized with a sterile rod. Subsequently, 25 g of each sample was weighed and 225 mL of 0.1% peptone water was added to prepare the first dilution. Subsequent serial dilutions were performed up to a dilution of 10^5 [9].

Although the eggshell is an important source of bacterial contamination, it was not analyzed due to limited diagnostic resources.

The protocol was made with six replicates.

2.4. Total Count of Coliforms and *E. coli*

The total coliform and *E. coli* counts were determined by the pour plate technique, using HiCrome Coliform Agar culture medium (HiMedia). One microliter of sample was diluted on Petri plates and approximately 15 mL of medium was added. The mixture was homogenized and after solidification, the plates were inverted and incubated at 35°C for 24 h [9]. Subsequently, the colonies were counted according to the instruction of the manufacturer: dark blue-violet colonies corresponded to *E. coli*, whilst, salmon-red colonies corresponded to coliforms. The results were plotted on a log scale as colony-forming units (CFU) g^{-1}.

2.5. Total Count of Mesophilic and Psychrotrophic Microorganisms

The pour plate technique was employed to count mesophilic and psychrotrophic microorganisms on Plate Count Agar medium (Merck). Briefly, 1 mL of sample was transferred to Petri plates, medium was added, and the mixture was homogenized. After solidification, the plates were inverted and incubated in a buffer at 35°C for 48 h to allow the growth of mesophilic bacteria. The plates were then maintained in a refrigerator (4°C) for seven days to allow the growth of psychrotrophic microorganisms. The colonies were counted [9] and the results are presented as log CFU g^{-1}.

2.6. Total Count of Molds and Yeasts

The molds and yeasts were counted by the spread plaque technique: 0.1 mL of

each diluted sample was transferred to Petri plates containing amalgamated and solidified Agar Sabouraud Dextrose culture medium (Merck), and spread with a sterile Drigalski spatula. The plates were then inverted and incubated at 20°C under biochemical oxygen demand conditions for five days. The colonies were counted [9] and the results are presented as log CFU g^{-1}.

2.7. Detection of *Salmonella* spp.

The 3M Petrifilm *Salmonella* Express system was employed to detect *Salmonella*. First, 25 g of egg sample was weighed and transferred to 225 mL of enriched broth prepared with Enrichment Base 3M *Salmonella* Express. The Supplement 3M *Salmonella* Express was homogenized and incubated at 41.5°C for 18 h. After incubation, 10 μL of the samples were spread with an inoculation spatula onto Petrifilm plates previously hydrated with 2 mL of distilled water. The plates were again incubated at 41.5°C for 24 h, and then the presumed *Salmonella* spp. colonies adhering directly to the film were read. The colony color ranged between red and brown, and included a yellow zone, gas bubbles, or both. The plates featuring colonies with the above characteristics were covered with a biochemical disc to confirm the presence of *Salmonella* spp. They were again incubated at 41.5°C for 4 h. A change from reddish-brown to a green-bluish hue, dark blue, or black indicated that the colonies were not *Salmonella* spp. The results are expressed as presence or absence of *Salmonella* spp.

The results were analyzed in accordance with the microbiological standards for food of animal origin that necessitates the absence of *Salmonella* spp. in a sample of 25 g for it to be considered safe [8].

2.8. Statistical Analysis

The SPSS software (SPSS Statistics for Windows, version 17.0; SPSS Inc., Chicago, IL) was employed for the statistical analysis. The descriptive analysis of microorganism counts and proportion analysis of qualitative variables, or rather, the results of the *Salmonella* test and the verification list, were performed. Student's *t*-test compared the microbiological profile of the eggs according to the commercialization site (town fairs and markets) and correlated the concentration of microorganisms with the variables in the verification list.

The continuous variables are expressed as mean ± standard deviation (SD), and the cut-off point for the *p*-value was 0.5% (level of statistical significance is $p < 0.05$).

3. Results and Discussion

Table 1 shows the results of the microbiological analysis of the samples from different municipalities, as well as the minimum and maximum rates of microbes in samples, according to the type of commercialization site.

The results of microbiological analyses did not show any statistical difference with regard to the commercialization site. Although the mean rate of total coliforms

Table 1. Microbiological characterization of egg samples from town fairs and markets.

Microorganism	Public fairs log CFU g^{-1}			Markets log CFU g^{-1}			*p*
	MIN	MAX	M	MIN	MAX	M	
Total coliforms	<1.0	4.72	1.85 ± 1.19	<1.0	4.52	1.75 ± 1.62	0.83
Escherichia coli	<1.0	3.70	1.33 ± 0.89	<1.0	3.48	1.32 ± 1.10	0.96
Mesophiles	<1.0	6.48	3.59 ± 1.69	1.48	7.12	3.40 ± 1.59	0.72
Psychrotrophs	<1.0	3.60	1.85 ± 0.91	<1.0	4.00	1.89 ± 1.01	0.88
Molds and yeasts	<1.0	6.60	3.76 ± 1.86	<1.0	7.20	3.31 ± 2.08	0.50
		Presence			Presence		
Salmonella		1 (2%)			0		

MIN, minimum rate in samples; MAX, maximum rate in samples; M, mean rate for municipalities; SD, standard deviation. Data are considered significant at *p* < 0.05.

was not very high, several samples reached the maximum rate, corresponding to 4.72 log CFU g^{-1} in town fairs. According to Franco and Landgraf [13], the total coliform rate of a specific type of food serves as an indicator of the hygienic condition of a site. Accordingly, egg samples with high coliform counts are considered to be placed in conditions lacking adequate hygiene.

In the present study, the mean *E. coli* counts were low, ranging between <1.0 and 3.70 log CFU g^{-1}. The amount of thermotolerant coliforms, represented mainly by *E. coli*, indicated fecal contamination [9]. Moreover, although Brazilian legislation does not have guidelines for mesophilic microorganisms in food products, the samples analyzed had high mean rates of these microorganisms. Similar rates were observed by Melo *et al.* [14], when they analyzed the hygienic and sanitary conditions of eggs from five producers in the town of Seropédica, RJ, Brazil. In the present study, the counts of mesophilic microorganisms in the samples from two suppliers ranged between 3.0 and 3.1 log CFU g^{-1}. This is of concern, because most pathogenic microorganisms grow at temperatures suitable for mesophiles [13].

Although there were no significant differences in the rate of psychrotrophic microorganisms among the samples obtained from town fairs and markets, the maximum rates indicated high levels in some samples. The psychrotrophic microorganisms indicate the general contamination of a product, level of hygiene employed during handling and storage, and conservation period [15].

In the present study, the count of molds and yeast in samples from town fairs and markets was 3.76 and 3.31 log CFU g^{-1}, respectively. The Decree No. 1/1990 specifies general norms for egg inspection and derivatives, and classifies eggs contaminated with fungi on the exterior and interior as unfit for consumption. Accordingly, these eggs are not safe for consumption and should not be sold [16].

Bacteria and fungi are the main microorganisms responsible for the physical and chemical changes observed in eggs. An assessment of the internal quality attributes of eggs associated with the storage form is indispensable; after oviposi-

tion, eggs inevitably begin to undergo structural changes, which can lead to the loss of quality and eventually deterioration. The reduction of contamination of equipment and facilities in farms can reduce bacterial contamination in final production of eggs by 80%. In this scenario, the presence of airborne fungi even with the adoption of Good Manufacturing Practices (GMPs) during egg production indicates the need for monitoring air quality in industries and greater selection and quality control of eggs coming from selected farms [8] [17] [18].

In the present study, among the samples analyzed for *Salmonella* sp., only one sample (3%) did not comply with the national standards [19]. This result is similar to that of previous studies, which have reported a 5% contamination with *Salmonella* in eggs [20] [21] [22].

According to a survey by Rossi and Bampi [23] on animal-derived products in the western and mid-western micro-regions of the state of Santa Catarina, Brazil, none of the 39 egg samples that underwent microbiological testing was contaminated with *Salmonella* sp.

Table 2 shows the results of the observation analysis on the hygienic and sanitary conditions of commercialized eggs. The percent of different types of commercial sites complying or not complying with the specific regulations is provided.

Only one (5%) fair presented at least one egg with cracks or a broken shell.

Table 2. Verification list for the commercialization of eggs at town fairs and markets.

Indicator	Town fairs (n = 18)		Markets (n = 18)	
	%C	%NC	%C	%NC
Cracks/broken shell	94	5.6	83	17
External dirt	6	94	11	89
External stains	0	100	11	89
Packaging	0	100	94	6
Place of shelf	0	100	0	100
Refrigeration	0	100	0	100
Closeness to other products	0	100	17	83
Expiration date	0	100	17	83
Mandatory information on packaging: "The consumption of this product in the raw or undercooked state may be harmful to health"	0	100	22	78
Mandatory information on package: "Keep eggs under refrigeration"	0	100	17	83
Inspection label	0	100	11	89
Presence of vectors and pests	78	22	94	6
Personal hygiene	0	100	NA	
Simultaneous handling of money and food	0	100	NA	
Total in each site (%)	9	92	32	69

C, conformity with the law; NC, non-conformity with the law; NA, not applicable.

Most commercialized eggs were intact, or rather, without any cracks. Markets had a 16% non-conformity rate with regard to this criterion. Pascoal *et al.* [24] reported low percent of breaks or cracks in commercialized eggs at different purchase sites (fairs, supermarkets, and farms) in Imperatriz, MA, Brazil, regardless of the type of purchase site. The cracks and breaks favor the penetration of bacteria and fungi, enabling contamination with harmful and pathogenic microorganisms [25] [26].

A high percent of eggs at town fairs and markets has been reported to be stained and contain external dirt. According to Barros *et al.* [27], such shortcomings are normally related to lack of hygiene, flaws in their maintenance, inadequate procedures from harvest to distribution, and exposure of eggs during sale. Pascoal *et al.* [24] also registered high rates of eggs with dirty shells commercialized in fairs (76%) and supermarkets (66%). These facts raise serious concern as dirty shells jeopardize the products' aspect and increase the probability of bacterial contamination.

No fair passed the conformity test with regard to the packaging in which the eggs were commercialized. The eggs were sold with inadequate packaging, such as nylon nets, newspapers, plastic bags, and others. The association between inadequate packaging and egg exposure to air currents or contaminating agents makes a product highly susceptible to harmful microorganisms [28].

No fair passed the conformity test with regard to the place where the eggs were exposed. In fairs, the eggs were placed in inappropriate containers, in dirty stalls, and on the floor, and even hung on bars. The eggs were commercialized along with other products, such as fruits, vegetables, cereals, meat, and condiments. When different products are placed close to eggs, their odors are transmitted and cross-contamination may occur.

No purchase site placed eggs under refrigeration. The eggs were maintained at room temperature; most were exposed to sun light and heat when sold at town fair sites without any coverage. Pascoal *et al.* [24] noted that, although refrigeration represented an important aspect of egg preservation, the commercialization process occurred without refrigeration. In fact, the introduction of microorganisms, such as *Salmonella* sp. increases in eggs subjected to fluctuations in temperature [29]. Decree RDC No. 35/2009 recommends that eggs should be refrigerated to avoid the growth and proliferation of microorganisms [12].

The above Decree, which deals with the consumption and preservation of eggs and inspection of their label, made certain information compulsory on the package. The expiry date on the packages was visible and complied with the norm only in the markets; the town fair sites did not mention it, making consumers' choice difficult. Only four and three markets complied with the requirement of mandatory information on the package regarding product consumption and refrigeration, respectively. Not all market sites complied with these requirements; the percent of compliance was 22% and 16%, respectively (Table 2).

Although this Decree [12] was published a long ago, most purchase sites still sell eggs without the mandatory information on the labels. Therefore, consumers are not informed whether the eggs are fresh, exposed to be surrounding for a long time, or deteriorating.

Fourteen purchase sites in town fairs and one in the markets analyzed did not pass the conformity test for vectors and pests. These insects jeopardize food safety, and are instrumental in transmitting several harmful microorganisms [10], dirt, and chemical contaminants from pest control agents [30].

Furthermore, food handlers in fairs failed to conform to the basic hygiene norms. There was lack of personal hygiene, and they were handling food and money simultaneously during the sale of products. Decree RDC n˚ 216/2004 specifies that no food handler should touch money or practice any activity, which may contaminate food [11].

Stringhini [31] evaluating eggs of commercial laying hens found that eggs not contaminated with *Pseudomonas aeruginosas* were not influenced by the storage temperature by the variable percentage of yolk. However, when submitted to experimental contamination, they presented worse values of percentage of yolk when stored at 28˚C. Therefore, the contamination worsened the quality of the yolk and the cooling was important in the maintenance of the physical characteristics of the egg, especially when it was contaminated.

All bacteria have their ideal environment where they find optimal growth conditions. Temperature may have positive or negative effects on the growth of a bacterial population. Because of this, it is worth mentioning that several bacteria grow at ambient temperatures varying from 25˚C to 40˚C (mesophilic), some live at temperatures below 15˚C (compulsory psychrophiles), there are facultative psychrophiles, which exhibit optimal growth below 20˚C and finally the thermophiles, which has its growth rate of 50˚C to 60˚C. According to Braun & Fehlhaber [32], the time of migration of a bacterium from the shell to the content depends on the storage temperature and degree of contamination, and at 30˚C it was one day and, at 7˚C, only after 14 days. Wang & Slavik [33] also found that the time interval between experimental bacterial contamination in the shell and its isolation on egg content was approximately three days when they were stored at 15˚C. According to Mendes [34] eggs stored in refrigeration showed better Haugh unit values regardless of contamination. With 10 days storage results showed that the refrigeration was able to maintain the internal quality of the egg even contaminated by *Pseudomonas aeruginosas*. The same author also reports that even eggs stored under refrigeration (5˚C) bacteria continued to multiply, but with less intensity in relation to eggs that were stored at 25˚C. Relative humidity during storage also interferes with egg quality, so many surveys recommend the ideal temperature for egg storage related to time and relative humidity. The recommended temperature for laying egg storage is between 8˚C to 15˚C, with a relative air humidity between 70% and 90% [31]. When storage exceeds 30 days, Baião and Cançado [35] recommend temperatures between 4˚C

to 12°C or near 0°C and for long periods, relative humidity of 70% and 80%. Storage between 0°C and 1.5°C with relative humidity of 85% and 90%, maintains the physical and microbiological quality of the egg for six months [36]. Souza & Souza [37] studying the storage time in relation to the storage temperature of quails eggs, observed that the refrigeration (8°C) maintained the internal quality of the eggs for 21 days of storage.

Table 3 shows the statistical difference ($p < 0.05$) characterizing the relationship between the observed hygienic and sanitary conditions and microbiological analysis results with respect to certain parameters in the verification list. We analyzed six groups of microorganisms and 14 sanitary parameters, and only total coliforms and three sanitary indicators exhibited correlation.

There was a significant ($p < 0.05$) relationship between the total coliform counts and the variables "external dirt", "external stains", and "closeness to other products", demonstrating a large number of non-conforming samples. No significant relationship was found for *E. coli* counts. Furthermore, no significant difference was detected with respect to the other variables.

The results of this study corroborate with those of previous studies [8] [17] [18].

The results of the present study reinforced that contaminated eggs are one of the common sources of infection through food products, increasing the chance of infections in consumers. While Brazilian Law necessitates the absence of *Salmonella* spp. in food products, these regulations are not uniformly enforced due to unofficial commerce of poultry products. In addition, the eggs sold at markets are often maintained at ambient temperature, which ranges from 27°C - 35°C throughout the year in Northeast Brazil. This temperature range is ideal for the proliferation of mesophilic bacteria, such as *Salmonella* spp. [8] [17] [18].

Eggshell cleaning is one of the most important steps to prevent microbiological contamination, because the major source of contamination of eggs is feces. However, even after being washed and sanitized, the eggs may be contaminated from external sources, which makes packaging, transportation, and storage critical factors for the quality of the final product [8] [17] [18].

The present observation analysis of egg selling sites revealed that they failed to comply of most the criteria established for egg commercialization and food handling, especially in town fairs. Although most samples proved to be microbiologically

Table 3. Correlation between the observational and microbiological analyses result of egg samples in town fairs and markets.

Variables	TC			*E. coli*		
	C	NC	*p*	C	NC	*p*
External dirt	<1.0	1.88	0.00	<1.0	1.36	0.13
External stains	<1.0	1.85	0.00	<1.0	1.35	0.13
Closeness to other products	<1.0	1.88	0.00	<1.0	1.36	0.13

C, conformity with the law; NC, non-conformity with the law; Data were considered significant at $p < 0.05$.

adequate according to the current legislation, one sample from a public fair was contaminated with *Salmonella* sp. The consumption of food contaminated with this bacterium is harmful for the population.

Moreover, all the samples revealed the presence of microorganisms, with most of them being pathogenic, such as *E. coli*, indicating unsatisfactory hygienic conditions and fecal contamination. This shows that the commercialization process requires more attention and supervision by the concerned authorities to ensure that high-quality food reaches consumers and the risks caused by micro-organisms are avoided.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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