ORIGINAL ARTICLE

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Detection of *Salmonella* spp. in eggs for human consumption from poultry farms in municipalities of Alagoas State, Brazil

Detecção de Salmonella *spp. em ovos destinados ao consumo humano de propriedades familiares avícolas em municípios do Estado de Alagoas, Brasil*

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ABSTRACT: Salmonellosis is a disease that worries poultry farmers and can seriously impact the food safety of the population that consumes products of animal origin. The present study sought to detect the presence of *Salmonella* spp. in eggs intended for consumption from family poultry farms in municipalities of Alagoas State. The study was carried out from eight farms, where the sample from each farm was obtained by making pools of shell and internal contents of six randomly selected eggs. The pools were submitted to microbiological culture and the colonies were characterized and evaluated by means of laboratory tests. To this end, 50% (4/8) of the shell samples and 75% (6/8) of the internal contents of the eggs were positive for *Salmonella* spp. In addition, *Klebsiella pneumoniae* 12.5% (1/8) and *Proteus* spp. 25% (2/8) were found in the shell samples, and *Yersinia* spp. 12.5% (1/8) in the internal contents of the eggs. *Salmonella* spp. and other enterobacteria were confirmed to occur in eggs intended for consumption. The way the birds were raised did not seem to have a significant influence on the results obtained, and the presence of passerines on the farms may have contributed to the existence of bacteria there. Being aware of the risk to public health that some of these bacteria can present, it is necessary to take decisions that support the small producer in search of food safety for all.

KEYWORDS: enterobacterium; salmonellosis; public health; food safety; toxinfection.

RESUMO: A Salmonelose é uma doença que preocupa os criadores de aves e pode impactar seriamente a segurança alimentar da população que consome produtos de origem animal. O presente estudo buscou detectar a presença de *Salmonella* spp. em ovos destinados ao consumo provindos de aviculturas familiares de municípios do Estado de Alagoas. O estudo foi realizado a partir de oito granjas, onde a amostra de cada uma delas foi obtida pela confecção de *pools* de casca e de conteúdo interno de seis ovos selecionados aleatoriamente. Os *pools* foram submetidos a cultivo microbiológico e as colônias caracterizadas e avaliadas por meio de testes laboratoriais. Para tanto, 50% (4/8) das amostras de casca e 75% (6/8) das amostras de conteúdo interno dos ovos foram positivos para *Salmonella* spp. Além disso, também fora constatada a presença de *Klebsiella pneumoniae* 12.5% (1/8) e *Proteus* spp. 25% (2/8) nas amostras de casca, e a presença de *Yersinia* spp. 12.5% (1/8) no conteúdo interno dos ovos. Foi confirmada a ocorrência de *Salmonella* spp. e outras enterobactérias em ovos destinados ao consumo. O modo de criação das aves não parece ter influenciado significativamente nos resultados obtidos e a presença de passeriformes nas granjas podem ter contribuído com a existência das bactérias no local. Tendo ciência do risco à saúde pública que algumas dessas bactérias podem apresentar, se faz necessária a tomada de decisões que apoiem o pequeno produtor em busca de uma segurança alimentar para todos.

PALAVRAS-CHAVE: enterobactérias; salmonelose; saúde pública; segurança alimentar; toxinfecção.

INTRODUCTION

The Brazilian poultry industry stands out for the production of meat and eggs for consumption, excellent sources of protein and greatly accessed by the general population, for their low cost and good quality (Aguiar, 2011). In the first three months of 2020 the production of chicken eggs in Brazil was 748 million dozen. This production was 0.1% higher than that recorded in the immediately preceding quarter and 6%

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higher than that recorded in the 1st quarter of 2019. In a comparative manner between the productions recorded in the first quarters of 2019 and 2020, it was observed that all regions showed an increase in production (IBGE, 2020).

The per capita consumption of eggs in Brazil is around 230 eggs per year (ABPA, 2019). The increased consumption of eggs by the population and the nutritional advantages of this food depend on the quality of the product offered to the consumer, which can influence its degree of acceptability in the market (Barbosa *et al.*, 2008). It is known that there is a fear of consuming eggs and egg-derived food due to probable contamination. Surveys of the Health Surveillance expose that from 2000 to 2015, 10.666 outbreaks, 209.240 patients, 2.095.354 exposed and 154 deaths by contaminated food were reported in Brazil, with 8.1% of these outbreaks caused by ingestion of eggs and egg-based products (BRASIL, 2016).

Data from the last agricultural and cattle raising sense reveals the amount of 4751 chicken eggs produced per year (one thousand dozen) by family producers in the state of Alagoas (IBGE, 2017). According to the Collegiate Directorate Resolution (RDC) 331/2019, the lists of microbiological standards for foods ready to be offered to consumers are defined. Among the determinations, with regard to the general requirements, foods must not contain pathogenic microorganisms, toxins or metabolites in quantities that cause harm to health. Thus, the sectors involved in the food production chain are responsible for carrying out periodic evaluations as to the adequacy of the process to meet the standards and determine the frequency of the analyses, ensuring that, during the entire validity period, the foodstuffs comply with the microbiological standards established, in accordance with the good manufacturing practices and other quality control programs.

Consumption habits of raw eggs, improper cooking, and the preparation of egg-based dishes without cooking are potential sources of infection by *Salmonella* spp. A single egg with *Salmonella enteritidis* can promote cross-contamination and contaminate large quantities of food, exposing a large number of consumers to the risk of developing severe disease (Braden, 2006).

Salmonella spp. is an enterobacterium that is not part of the intestinal flora of animals, and its presence is a major indicator of infection (Merino and Lösch, 2006). It acts as a major cause of foodborne infections, acting in a cosmopolitan manner, and eggs are among the most important vehicles of the pathogen for humans. The symptoms often reported in *Salmonella* spp. infections in humans are abdominal cramps, vomiting, fever and diarrhea, however, the picture in children, elderly and immunosuppressed people can worsen and lead to consequences such as pneumonia, sepsis and death (Campello, 2012).

Birds become infected by *Salmonella* spp. by ingesting the bacteria through contaminated soil, water and food, eventually colonizing the intestine and migrating through macrophages to ovaries and oviducts (Gantois *et al.*, 2009). In poultry farming, *Salmonella* spp. is considered a threat, either by vertical transmission to future generations of birds or by contamination of chicken meat and eggs intended for consumption (Barancelli, 2012). Besides the transovarian route as a form of egg contamination, there is contact with feces from the cloaca and contaminated environments after laying (Stringhini *et al.*, 2009).

According to the Ministry of Agriculture, Livestock and Supply, whole eggs must present a maximum standard count of 5×10^4 CFU/g, absence of fecal coliforms and *Sthaphylococcus aureus* in 1g of egg and the absence of *Salmonella* spp. in 25g of egg, where the presence of *Salmonella* spp. in the food makes it unfit for consumption (BRASIL, 1990).

Considering the potential risk that this contaminated food represents for public health and the lack of this type of study in the state of Alagoas, the present study was carried out with the objective of investigating the presence of *Salmonella* spp. in the shell and internal contents of chicken eggs from subsistence poultry farms in municipalities of the State of Alagoas.

MATERIALS AND METHODS

The study was conducted in eight farms in the state of Alagoas, four located in the immediate geographic region of Palmeira dos Índios, two in Serrana dos Quilombos, and two in São Miguel dos Campos. Palmeira dos Índios belongs to the intermediate geographic region of Arapiraca, while Serrana dos Quilombos and São Miguel dos Campos are part of the intermediate geographic region of Maceió (Table 1).

The number of farms selected for the study was determined from a proportion calculation in Openepi (Dean et al., 2019). For the calculation, a number of 4751 and family poultry farms present in the state of Alagoas was considered (IBGE, 2017). With a precision of 10%, a confidence interval of 95% and a predicted frequency of 2.36%, the sample value n=8 was obtained (Wolschick and Bosco, 2014). Twelve eggs were collected per establishment, the minimum quantity provided by

Table 1. Division of municipalities into immediate and intermediate geographic regions with investigated farms in the State of Alagoas. IBGE, 2019.

Municipalities	Number of farms	Immediate region	Intermediate region
Coruripe	2	São Miguel dos Campos	Maceió
União dos Palmares	2	Serrana dos Quilombos	Maceió
Mar Vermelho	2	Palmeira dos Índios	Arapiraca
Paulo Jacinto	2	Palmeira dos Índios	Arapiraca

the farms, totaling 96 eggs. The eggs were randomly selected in the farms' warehouse, selecting white and red eggs without distinction, with one day of lay, from birds of indeterminate strains. After field collection, the eggs were transported to the laboratory of Infectious Diseases at the Federal University of Alagoas, stored in packages provided by the producer and kept at room temperature for analysis.

For the analyses, six eggs were randomly selected to obtain pools of shell and contents, which were used separately to obtain two distinct pools. Aseptically, 25g of shell and 25g of internal content - yolk and white - were weighed on precision scales (BRASIL, 1990a). The weighed material was placed in erlenmayers with 225 ml of peptone water and placed in a bacteriological oven at 37°C for 24 hours, so that all bacteria present in the samples had minimal growth and could be seen in the subsequent analyses. Then, 1ml aliquots were transferred to test tubes containing 9ml of selective enrichment broth for enterobacterium tetrationate and incubated at 37°C for 24 hours. Surface seeding was performed from each broth on MacConkey agar and EntericHektoen agar, and these plates were incubated at 37°C for 24h. After the incubation time and bacterial growth, the colonies with morphology typical of *Salmonella* spp. and other pathogens of interest to public health were submitted to phenotypic characterization through biochemical tests in order to observe not only *Salmonella* spp. but also other enteropathogens such as *Proteus* spp., *Klebsiella* spp., among others.

According to the Manual of Clinical Microbiology of the National Health Surveillance Agency (2004), the biochemical tests used allow more than 95% of *Salmonella* spp. to be differentiated from other culturable enterobacteria at the genus level, acting as an efficient confirmatory method for food contamination by pathogenic bacteria (Farmer, 1991). These tests consisted of: Simmons' Citrate (CIT), to verify the use of citrate as a carbon source; Lysine (LIA), to verify the decarboxylation of lysine; SIM medium, to verify motility, H2S production and indole production; Triple Sugar Iron Agar (TSI), the main medium that differentiates *Salmonella* spp. from other enterobacteria with gas formation and fermentation of sugars (lactose, sucrose, and glucose); Methyl Red (VM), to observe glucose fermentation via the mixed acid pathway; Voges-Proskauer (VP), to observe glucose fermentation via the butylene glycol pathway; and Urease to observe the bacteria's ability to degrade urea (Andrews et al, 2016). The media and broths used in the isolation and biochemical characterization phases came from HiMedia Laboratories.

The interpretation of the tests was performed with the aid of the reference table on identification of Enterobacteriaceae by biochemical tests. The data obtained were tabulated in EXCEL® and submitted to statistical analysis (Farmer, 1991). The statistical analysis consisted of studying the absolute and relative frequencies.

RESULTS AND DISCUSSION

The results of the bacteriological analyses revealed that of the eight shell samples, seven had vivaciously growing colonies, as had the analyses of the internal contents of the eggs, where of the eight samples investigated, seven had formed colonies.

The grown colonies were submitted to macroscopic analysis and 100% presented colonial characteristics of enterobacterium: smooth, convex, circular, and shiny. Colonies suggestive of *Salmonella* spp. grew on Hektoen agar, which, due to the production of H2S by these colonies, were blackened. On MacConkey agar, due to the absence of H2S production by *Salmonella* spp. on this medium, colonies that were transparent were considered suggestive. On MacConkey agar, fermentation of lactose by fermenting bacteria could also be noted. The colonies were then submitted to morphotyping evaluation under light microscopy, and all presented as Gram-negative bacillus.

After being submitted to biochemical tests, 57% (4/7) of the bacterial colonies from the shell samples were positive for *Salmonella* spp., while the percentage obtained in the internal content samples was 86% (6/7). Considering the total number of farms subjected to the experiment, 50% (4/8) of the shell samples and 75% (6/8) of the internal content samples were positive for *Salmonella* spp. This demonstrates that at least one type of sample collected per farm had the presence of the pathogen, and points to the fact that the eggs produced by these farms contain the bacteria, either by infection of the birds or by contamination through the feces. This information poses an imminent risk of bacterial infections to the end consumers of these eggs (Eddin *et al.*, 2019). In addition, *Klebsiella pneumoniae* 12.5% (1/8) and *Proteus* spp. 25% (2/8) had also been found to be present in the shell samples. In the internal contents of the eggs, *Yersinia* spp. 12.5% (1/8) was detected, another important pathogen that is not part of the natural microbiota of birds and the environment and brings important risks to public health (Feddern *et al.*, 2017). The test results for the shell samples are expressed in Table 2 and the results for the internal content samples in Table 3.

Reports of lower or even non-existent levels of contamination are frequent in the literature, as in the research of *Salmonella* spp. in chickens raised in backyard and eggs sold in open fairs in the city of Fortaleza-CE, performed by Gomes Filho *et al.* (2014), where no positive sample was observed. The same happened in the work of Campello (2012) in São Paulo with 1.47% of white eggs positive for *Salmonella* spp., of Wolschick and Dal Bosco (2015) who obtained positivity of 2.36% in farm eggs located in Rio Grande do Sul and of Perdoncini *et al.* (2011) with 2.32% positivity in poultry establishments located in Santa Catarina. Higher levels of contamination in proven literature are described by Oliveira and Silva (2000) in Campinas-SP with 9.6% positivity in shells and 3.2% in yolks, and by Andrade *et al.* (2004) with 4.46 % of eggs sold in open fairs in Goiânia-GO. There are no studies

Table 2. Biochemical analyses of the shell samples.

A	CIT	LIA	SIM	TSI	VM	VP	U	Enterobacterium
1	-	+	---	++++	-	+	-	*K. pneumoniae*
2	+	+	+-+	++--	+	-	-	*Salmonella* spp.
3	+	+	+-+	++--	+	-	-	*Salmonella* spp.
4	+	+	+-+	++--	+	-	-	*Salmonella* spp.
5	+	+	+-+	++--	+	-	-	*Salmonella* spp.
6								
7	+	-	+++	++-+	+	+	+	*Proteus* spp.
8	+	-	+++	++-+	+	+	+	*Proteus* spp.

A. Farm samples. CIT. Citrate. LIA. Lysine. SIM. H_2S, indole e motility. TSI. Gas, glucose, lactose and sucrose. VM. Methyl Red. VP. Voges-Proskauer. U. Urease. (+) positive. (-) negative.

Table 3. Biochemical analyses of the internal content samples.

A	CIT	LIA	SIM	TSI	VM	VP	U	Enterobacterium
1	+	+	+-+	++--	+	-	-	*Salmonella* spp.
2	+	+	+-+	++--	+	-	-	*Salmonella* spp.
3								
4	+	+	+-+	++--	+	-	-	*Salmonella* spp.
5	-	-	-++	----	+	-	+	*Yersinia* spp..
6	+	+	+-+	++--	+	-	-	*Salmonella* spp.
7	+	+	+-+	++--	+	-	-	*Salmonella* spp.
8	+	+	+-+	++--	+	-	-	*Salmonella* spp.

A. Farm samples. CIT. Citrate. LIA. Lysine. SIM. H_2S, indole e motility. TSI. Gas, glucose, lactose and sucrose. VM. Methyl Red. VP. Voges-Proskauer. U. Urease. (+) positive. (-) negative.

of this type in the state of Alagoas, and the data presented are innovative in the sanitary representation of local family farms.

During the collection, it was found that half of the farms (4) used intensive housing, where birds were kept in cages designed to provide protection against possible predators, thermal comfort and reduce the transmission of pathogens, internal parasites and external parasites. The other farms (4) used the semi-intensive type of farming, where the birds had access to the outside of the barn, but did not enjoy quality green matter, nor did they have areas of natural shade to protect them from insolation. On these semi-intensive farms, eggs were seen laid outside the nests on the litter. This type of information reveals the point that the farming method did not significantly influence the results obtained, not necessarily attributing the occurrence of *Salmonella* spp. on farms to environmental contamination outside the cages (Gantois et al., 2009).

The presence of wild birds on the farms was noted, especially during the provision of food and water for the poultry. It was also noted that the feeding of the birds was different, with those with intensive type of farming based on commercial feed, and those with semi-intensive system based on corn. The owners were raising the chickens not only for the purpose of marketing the eggs, but also for their own consumption. This information supports the idea that the type of poultry feed did not influence the *Salmonella* spp. results on the farms, but the presence of wild birds interspecifically related to domestic poultry may have influenced the positivity of the farms. According to Da Silva *et al.*, (2014), wild species, especially passerines, are considered the main carriers of these types of pathogens, and their transmission to domestic birds can occur through contact of contaminated feces with the environment, water and food.

It is necessary for government institutions to invest in supporting these families, who produce for their own subsistence, helping in a safer production for the consumer and in greater effectiveness in production, without further losses in the lives of the producing families (Jones *et al.*, 2018).

The risk to public health is imminent, with the possibility of disease outbreaks in the local population and costs to the public health system. It is important to emphasize that egg producers for commercial purposes have the responsibility to provide harmless food to the final consumer. It is suggested, therefore, the help of municipalities to implement programs for the control of pathogenic microorganisms in farms and the need for actions for the proper education of poultry handlers and breeders, as well as traders and consumers, aiming at better hygienic-sanitary conditions of the product, to preserve its final quality and the

health of the population. Being aware of the risk to public health, it is necessary to make decisions that support the small producer so that it is possible to achieve food safety for all (França *et al.*, 2022).

CONCLUSIONS

Salmonella spp. and other enterobacteria were found in eggs for consumption, both in the shell and in the internal contents. *Yersinia* spp. was detected in the internal content of eggs from one of the farms sampled. The way the birds were raised does not seem to have a significant influence on the results obtained and the presence of passerines on the farms may have contributed to the existence of bacteria there. It is necessary to prevent the existence of external passerines on the farm, as well as to maintain a periodic sanitary control of the establishment in order to find possible foci of contamination within the property, and thus minimize contamination of the environment and infection of the animals. The risk to public health is imminent, with the possibility of disease outbreaks in the local population and costs to the public health system. It is important to invest in support for small producers, in order to increasingly seek to improve their production and ensure the health of a large part of the population.

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