

Prevalence And Levels Of *Salmonella* In Commercial Shell Eggs From Santander, Colombia

Prevalencia Y Niveles De *Salmonella* En Huevos Con Cáscara Comerciales De Santander, Colombia.

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ABSTRACT

Salmonellosis remains one of the leading foodborne diseases worldwide, and eggs are recognized as an important vehicle of *Salmonella*, particularly *Salmonella enterica* subsp. *enterica* serovar Enteritidis. This study aimed to determine the prevalence and load of *Salmonella* in commercial shell eggs produced in Santander, Colombia. A total of 360 egg samples were collected from six provinces over a three-month period.

Eggshells and internal contents were analyzed separately following ISO 6579-1:2017, with biochemical and serological confirmation of isolates. *Salmonella* was detected in five samples, resulting in an overall prevalence of 1.4%. Four positive samples were found on eggshells and one in egg contents, originating from two farms. One isolate was presumptively identified as *S. Enteritidis*, corresponding to a prevalence of 0.3%. Quantification using the most probable number method showed an average concentration of 0.052 MPN/g. The detection of *Salmonella* indicates a potential public health risk, particularly when eggs are consumed raw or undercooked. These findings establish baseline data on the presence of *Salmonella* in commercial eggs from Santander and highlight the importance of maintaining microbiological surveillance and strengthening biosecurity and sanitary control measures to ensure egg safety.

Key words: *Food safety; prevalence; Salmonella Enteritidis; Salmonella; shell eggs.*

RESUMEN

La salmonelosis es una de las principales enfermedades transmitidas por alimentos a nivel mundial, y los huevos son reconocidos como un vehículo de *Salmonella* especialmente *Salmonella enterica* subsp. *enterica* serovar Enteritidis. El objetivo de este estudio fue determinar la prevalencia y la carga de *Salmonella* en huevos comerciales producidos en el departamento de Santander, Colombia. Se recolectaron 360 muestras de huevos provenientes de seis provincias durante un período de tres meses. Las cáscaras y los contenidos internos se analizaron por separado siguiendo el método ISO

6579-1:2017, con confirmación bioquímica y serológica de los aislamientos. *Salmonella* se detectó en cinco muestras, correspondiente a una prevalencia de 1,4 %. Cuatro muestras positivas se encontraron en la cáscara y una en el contenido del huevo, provenientes de dos granjas. Un aislamiento fue identificado presuntamente como *S. Enteritidis*, con prevalencia de 0,3 %. La cuantificación mediante el método del número más probable mostró promedio de 0,052 NMP/g. Aunque la prevalencia y las cargas bacterianas fueron bajas, la detección de *Salmonella* evidencia un potencial riesgo para la salud pública, especialmente cuando los huevos se consumen crudos o poco cocidos. Los resultados obtenidos establecen una línea base sobre la presencia de *Salmonella* en huevos comerciales de Santander y resaltan la importancia de mantener la vigilancia microbiológica y fortalecer las medidas de bioseguridad y control sanitario para garantizar la inocuidad del producto.

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Palabras clave: cáscara de huevo, prevalencia, *Salmonella* Enteritidis, *Salmonella*, inocuidad alimentaria

INTRODUCTION

Salmonellosis is a major foodborne disease of global public health concern and remains one of the leading causes of bacterial gastroenteritis worldwide. Non-typhoidal *Salmonella* is commonly associated with the consumption of contaminated foods of animal origin, particularly poultry products and shell eggs (CDC, 2020; FDA, 2022; OMS, 2018). In the United States,

Salmonella enterica serovars Enteritidis and *S. Typhimurium* account for a substantial proportion of human salmonellosis cases and are frequently linked to eggs and egg products (USDA, 2023).

Shell eggs may become contaminated with *Salmonella* Enteritidis through several transmission routes. Vertical transmission occurs when the pathogen colonizes the

reproductive organs of laying hens, allowing contamination of egg contents prior to shell formation. Horizontal transmission may occur during oviposition or post-lay handling, as eggs pass through the cloaca and become exposed to fecal material, dust, water, equipment, or handlers. Under favorable conditions of temperature, humidity, and storage time, *Salmonella* may penetrate the eggshell and reach the internal contents, increasing the risk of human exposure (Kaiser & Lamont, 2001).

Eggs are widely consumed worldwide due to their affordability and high nutritional value, providing high-quality protein, vitamins, and essential minerals. However, the consumption of raw or undercooked eggs, as well as egg-based products that do not undergo sufficient heat treatment—such as mayonnaise, salad dressings, desserts, and beverages—has frequently been associated with outbreaks of salmonellosis (ICMSF, 2016). Consequently, shell eggs remain a critical control point within food safety systems.

Colombia is one of the main egg-producing countries in Latin America, and the department of Santander represents an important contributor to national production.

In 2023, egg production in Santander reached approximately 3.878 billion units (FENAVI, 2024). Nationally, egg production totaled 16.864 billion units during the same year (FENAVI, 2024). Nevertheless, the sector experienced a production decrease of approximately 4.6% in 2022, reaching levels similar to those reported in 2020, largely due to increased production costs associated with key feed inputs such as corn and soybean (Bolsa Mercantil, 2024).

Shell eggs are a food of high biological value, yet their handling and origin can jeopardize public health;" *The monitoring of hygienic-sanitary conditions is critical not only in primary production but also in the food services where these raw materials are processed, as deficiencies in the environment can enhance the transmission of pathogens*" (Castillo-Luquez et al., 2023). In Colombia, the prevalence of foodborne diseases is closely linked to;"*food security in vulnerable populations, where access to safe food is a fundamental pillar in preventing malnutrition diagnoses*" (Mora-Aguirre, et al., 2024a; Mora-Aguirre et al., 2024b).

Globally, egg consumption has increased by approximately 12%. Regarding international trade, the Netherlands ranks as the leading

exporter with 21.7% of global exports, followed by the United States with 11.7% (Bolsa Mercantil, 2024). In Colombia, approximately 98% of poultry farms correspond to small-scale family production systems with limited technological development, while only 2% are highly technified. Egg-laying production accounts for approximately 39% of poultry activities, primarily concentrated in the departments of Cundinamarca and Santander, followed by Valle del Cauca, Antioquia, and Quindío.

Eggs are considered one of the most regulated animal-derived food products in international trade due to strict sanitary and safety requirements. According to the Food and Agriculture Organization, global egg production has doubled since the 1960s, reflecting its growing importance as an accessible and nutritionally valuable food source (FAO, 2023). Eggs are also considered one of the most affordable sources of high-quality protein available in the market. In Colombia, the average consumption is estimated at approximately 291 eggs per person per year (Rey-Rodríguez, et al., 2023; Soto & Caballero, 2021; Agronegocios, 2020).

Public health authorities, including the Centers for Disease Control and Prevention, the Pan American Health Organization, and the Colombian Ministry of Health and Social Protection, have classified eggs as a high-risk food for public health due to their association with salmonellosis transmission (CDC, 2020; Resolution 0719 of 2015). Salmonellosis remains a significant global health problem, causing approximately 2.2 million deaths worldwide, with children representing the most vulnerable population (OMS, 2018). In the United States alone, the CDC estimates approximately 1.35 million cases of salmonellosis annually, resulting in about 420 deaths (FDA, 2023).

The National institutions such as the National Federation of Poultry Producers (FENAVI) and the Colombian Agricultural Institute (ICA) are currently working toward strengthening these standards (Moreno, 2019).

Salmonella is one of the most important pathogens responsible for foodborne diseases. The genus includes more than 2,400 serotypes, among which *Salmonella* Enteritidis and *Salmonella* Typhimurium are the most frequently associated with human infections, accounting for nearly 50% of

reported cases in the United States (USDA, 2019).

According to the World Health Organization, foodborne diseases affect approximately one in ten people worldwide each year, causing around 420,000 deaths annually. Children under five years of age represent nearly one-third of these fatalities (WHO, 2025). Non-typhoidal *Salmonella* is one of the principal etiological agents responsible for these illnesses. Furthermore, *Salmonella* is recognized as one of the four leading causes of diarrheal diseases worldwide (Peña-Rivera, et al., 2023; OMS, 2018). The bacterium is highly resilient and can survive for several weeks in dry environments and for months in water.

In the United States, the Food and Drug Administration has estimated that approximately 79,000 foodborne illness cases are associated with the consumption of contaminated eggs each year, resulting in approximately 30 deaths (FDA, 2017). In Europe, *Salmonella* is responsible for nearly one-third of reported foodborne outbreaks. In 2023, salmonellosis ranked as the second most frequently reported foodborne disease in the United States, but it remained the

leading cause of foodborne hospitalizations and deaths (FDA, 2023).

In Colombia, the National Institute of Health reported 719 foodborne disease outbreaks during 2023 associated with contaminated food and water. Among the identified etiological agents were *Escherichia coli* (5.4%), *Staphylococcus aureus* (3.9%), *Salmonella* (1.5%), and *Bacillus cereus* (1.3%). Most outbreaks occurred in households (49.7%), followed by educational institutions (13.9%) and restaurants (10.7%). The most affected age groups were individuals between 20 and 49 years (42.1%), adolescents between 10 and 19 years (27.1%), and children aged 1 to 4 years (11.6%) (INS, 2024).

Previous studies conducted in Colombia have also demonstrated the presence of *Salmonella* in egg samples. For example, (Castañeda et al., 2017) detected *Salmonella* in 9 (9.4%) of eggs collected from four localities in Bogotá, with 5 isolates found in the internal egg contents and 4 on the eggshell surface.

Therefore, the objective of this study was to determine the prevalence and concentration of *Salmonella* in commercial shell eggs produced in Santander, Colombia. The

findings provide baseline information to support food safety risk management,

improve control programs in the egg production chain, and protect public health.

MATERIALS AND METHODS

Study area and sample collection. The study was conducted in the department of Santander, Colombia, a major egg-producing region at the national level. According to official records from the Colombian Agricultural Institute (ICA), Santander has both registered commercial laying-hen farms operating under biosecurity programs and unregistered farms for which no official census is available.

A total of 360 commercial shell eggs were collected from six of the seven provinces of Santander (Guanentá, Comunera, Yariguíes, García Rovira, Metropolitana, and Vélez). Samples were obtained from six commercial farms, which were coded as farms A through F to ensure confidentiality, as recommended by the National Poultry Federation of Colombia (FENAVI).

Eggs were collected over a three-month period between November 15, 2022, and March 2023, with nonconsecutive sampling days. Eggs originated from flocks aged approximately 29 to 80 weeks and were collected from houses using either manual or

automatic egg collection systems. Samples were taken primarily from the morning lay (5:00 to 6:00 a.m.) and, in some cases, from the evening lay (5:00 to 6:00 p.m.). For each farm and sampling month, 20 eggs were randomly selected from three trays, resulting in approximately 60 eggs per farm and a total sample size of 360 eggs.

Sample preparation. Eggshells and internal contents were analyzed separately. Eggs were aseptically cracked using sterile spatulas, and the shells were separated from the combined albumen and yolk. Each fraction was placed into sterile bags. From each sample, 25 g were used for microbiological analysis, while the remaining portions were stored at refrigeration temperature as retain samples for subsequent quantification when applicable.

Detection of *Salmonella*. The presence or absence of *Salmonella* was determined according to ISO 6579-1:2017, in compliance with Colombian Technical Standard NTC 1240 (2011) and Resolution 1407 (2022). Detection consisted of pre-enrichment,

selective enrichment, isolation on selective agar media, and biochemical and serological confirmation.

For pre-enrichment, 25 g of sample were added to 225 mL of buffered peptone water (Oxoid, UK) and incubated at 37°C for 18 h. Following pre-enrichment, 0.1 mL of culture was transferred to 10 mL of Rappaport–Vassiliadis soya broth (RVS; Merck, Germany) and incubated at 41.5°C ± 1°C for 24 h ± 3 h. In parallel, 1 mL of pre-enrichment culture was transferred to 10 mL of Müller–Kauffmann tetrathionate-novobiocin broth (MKTTn; Merck) and incubated at 37°C ± 1°C for 24 h ± 3 h.

Selective isolation was performed by streaking enriched cultures onto xylose lysine deoxycholate (XLD) agar and Hektoen enteric agar (Oxoid). Plates were incubated at 37°C ± 1°C for 24 h ± 3 h. Presumptive *Salmonella* colonies were selected based on typical morphology: red colonies with or without black centers on XLD agar and blue-green colonies with or without black centers on Hektoen agar.

Biochemical and serological confirmation

At least three presumptive colonies per plate were subjected to biochemical identification using the API 20E system (bioMérieux, France), following the manufacturer's instructions. Results were interpreted using the API 20E database software. Additional biochemical tests, including oxidase activity and carbohydrate fermentation, were used to exclude non-*Salmonella* isolates.

Serological confirmation was performed by slide agglutination using commercial O (somatic) and H (flagellar) antisera (Thermo Scientific™, USA; Oxoid). Confirmed reactions were interpreted according to the Kauffmann–White scheme. A reference strain of *Salmonella* Enteritidis (ATCC 13067) was used as a positive control.

Quantification of *Salmonella* spp.

Samples confirmed positive for *Salmonella* spp. were subjected to quantitative analysis using the most probable number (MPN) method, following the Food Safety and Inspection Service (FSIS–USDA) Microbiology Laboratory Guidebook, Appendix 2.05d. Analyses were performed in triplicate with a 95% confidence level.

A homogenized sample (65 ± 2 g) was mixed with 585 mL of RVS broth and processed in

a stomacher for 2 min. Serial dilutions corresponding to 10 g, 1 g, and 0.1 g were prepared and inoculated into three tubes per dilution. Tubes were incubated at 41°C for 24 h, and growth was assessed by turbidity.

Positive tubes were confirmed by plating on XLD agar and by biochemical tests using triple sugar iron (TSI) and lysine iron agar (LIA). MPN values were calculated using FSIS–USDA reference tables.

RESULTS AND DISCUSSION

Prevalence of *Salmonella* in shell egg samples

A total of 360 commercial shell eggs were analyzed to determine the presence of *Salmonella* Eggshells and internal contents

(albumen–yolk) were evaluated separately. *Salmonella* was detected in five samples, resulting in an overall prevalence of 1.39%. Of the positive samples, four corresponded to eggshells (1.11%) and one to egg contents (0.27%) (Table 1).

Table 1. Presence of *Salmonella* in shell eggs collected from six commercial farms in Santander, Colombia

Farm	Egg samples analyzed	shell Egg positive (n)	shell Egg prevalence (%)	shell Egg samples analyzed	Egg content Egg positive (n)	Egg content Egg prevalence (%)
A	60	1	1.7	60	1	1.7
B	60	0	0.0	60	0	0.0
C	60	3	5.0	60	0	0.0
D	60	0	0.0	60	0	0.0
E	60	0	0.0	60	0	0.0
F	60	0	0.0	60	0	0.0
Total	360	4	1.1	360	1	0.3

The present study provides baseline data on the prevalence and concentration of *Salmonella* in commercial shell eggs produced in Santander, Colombia. The overall prevalence of 1.4% observed in this study is relatively low compared with reports

from other regions in Latin America, where prevalence values ranging from 2.4% to more than 15% have been documented in shell eggs and egg contents (Freije et al., 2019; Rumão et al., 2020; Guier et al., 2022). These differences may reflect variability in

biosecurity practices, flock management, environmental conditions, and regulatory oversight across production systems.

Positive samples were identified in eggs collected from two farms (A and C), whereas no *Salmonella spp.* was detected in samples from farms B, D, E, or F. Farm C showed the highest prevalence on eggshells (5.0%), while farm A presented *Salmonella spp.* in both eggshell and internal content samples, each with a prevalence of 1.7%.

The detection of *Salmonella* primarily on eggshells rather than in internal contents suggests that post-lay contamination and environmental exposure are likely the dominant routes of contamination in the studied farms. Similar findings have been reported in studies conducted in Colombia and other countries, where eggshell contamination was more frequent than internal contamination (Ramírez et al., 2015; Castañeda et al., 2017).

Factors such as contact with fecal material, contaminated surfaces, dust, and handling practices during collection and storage may facilitate shell contamination, while subsequent bacterial penetration into the egg contents can occur under conditions of high

humidity, elevated temperature, and extended storage time.

Presumptive identification of *Salmonella* Enteritidis. Biochemical identification using the API 20E system confirmed five *Salmonella* isolates. One isolate from farm C was identified as *Salmonella* Gallinarum and was excluded from further analysis. The remaining four isolates were confirmed as *Salmonella* by biochemical testing.

Serological analysis revealed that one isolate reacted positively with both O (somatic) and H (flagellar) antisera and was presumptively identified as *Salmonella* enterica serovar Enteritidis. This result corresponds to a prevalence of 0.27% among the total egg samples analyzed (Table 2).

Table 2. Prevalence of presumptive *Salmonella* Enteritidis in commercial shell eggs

Sample type	Total samples	Presumptive <i>S. Enteritidis</i> (n)	Prevalence (%)	95% CI (%)
Shell eggs	360	1	0.3	0.93

Only one isolate was presumptively identified as *Salmonella enterica* serovar Enteritidis,

representing a prevalence of 0.3%. Although low, the presence of this serovar is of particular concern due to its strong association with egg-related outbreaks of salmonellosis worldwide.

International surveillance data from the United States and the European Union

consistently identify *S. Enteritidis* as one of the leading causes of egg-associated salmonellosis, even in countries with established control programs (CDC, 2023; Stiliz et al., 2022). Therefore, the detection of this serovar in commercial eggs from Santander, even at low prevalence, highlights the need for continued vigilance.

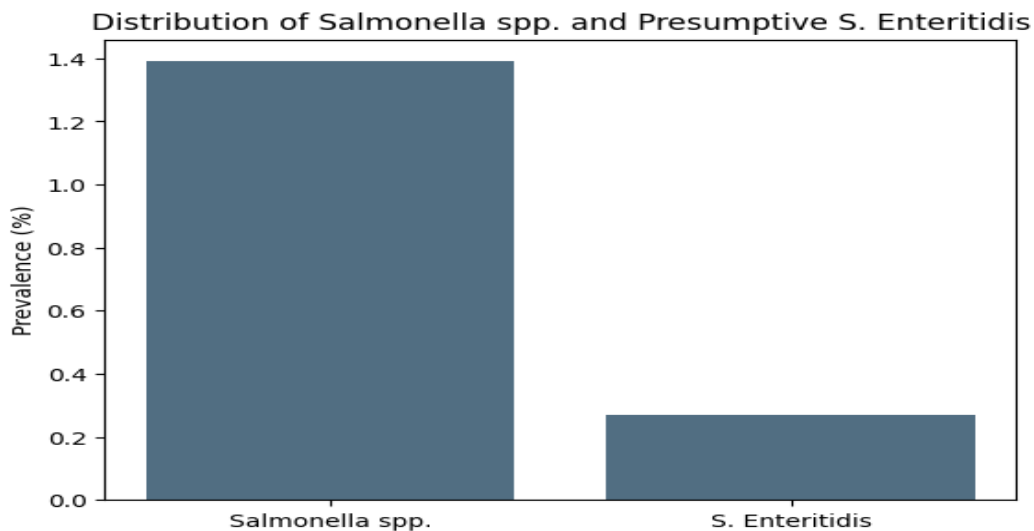


Figure 1. Distribution of *Salmonella* and presumptive *S. Enteritidis* among positive samples.

In addition, Xu et al. (2022) conducted a study to corroborate the relationship between the virulence of *Salmonella enterica* serovar Enteritidis and egg yolk as an inoculation medium, administering the bacterium to mice, which resulted in a high mortality rate.

Therefore, it is crucial to implement sanitary control measures in egg-producing farms in

Santander and throughout Colombia, especially those that have not been registered with governmental authorities.

Quantification of *Salmonella* by MPN

Quantitative analysis was conducted on samples confirmed positive for *Salmonella* using the most probable number (MPN) method. The average concentration of

Salmonella among positive samples was 0.052 MPN/g. Samples from farm A showed an average of 0.042 MPN/g, whereas

samples from farm C presented a higher average concentration of 0.067 MPN/g (Table 3).

Table 3. Quantification of *Salmonella* in positive shell egg samples by the MPN method

Farm	10	1	0,1	Reported positive values	Estimated NMP/g	95% Confidence Limit
AM1	1/3	1/3	0/3	110	0.074	0.42
AM1	1/3	0/3	0/3	100	0.036	0.18
AM1	0/3	0/3	0/3	000	<0.03	0.095
AM2	1/3	0/3	0/3	100	0.036	0.18
AM2	0/3	0/3	0/3	000	<0.03	0.095
AM2	0/3	0/3	0/3	000	<0.03	0.095
AM1	1/3	0/3	0/3	100	0.036	0.18
AM1	0/3	0/3	0/3	000	<0.03	0.095
AM1	0/3	0/3	0/3	000	<0.03	0.095
CM1	1/3	1/3	0/3	110	0.074	0.2
CM1	0/3	0/3	0/3	000	<0.03	0.095
CM1	0/3	0/3	0/3	000	<0.03	0.095
CM2	2/3	0/3	0/3	200	0.092	0.38
CM2	0/3	0/3	0/3	000	<0.03	0.095
CM2	0/3	0/3	0/3	000	<0.03	0.095
CM3	1/3	0/3	0/3	100	0.036	0.18
CM3	0/3	0/3	0/3	000	<0.03	0.095
CM3	1/3	0/3	0/3	100	0.036	0.18
Media					0.052	0.15

Across all positive samples, detectable *Salmonella* levels were observed in approximately 33% of the replicates, indicating low but measurable contamination levels.

The average *Salmonella* concentration of 0.052 MPN/g observed in this study indicates low-level contamination. Nevertheless, low bacterial loads do not eliminate public health risk, particularly when eggs are consumed raw or undercooked or are used as

ingredients in foods that do not receive adequate heat treatment. Previous risk assessments have demonstrated that even small numbers of *Salmonella* cells can lead to illness, depending on host susceptibility

and consumption patterns (ICMSF, 2016). This is especially relevant in regions where traditional foods and beverages prepared with raw eggs are commonly consumed.

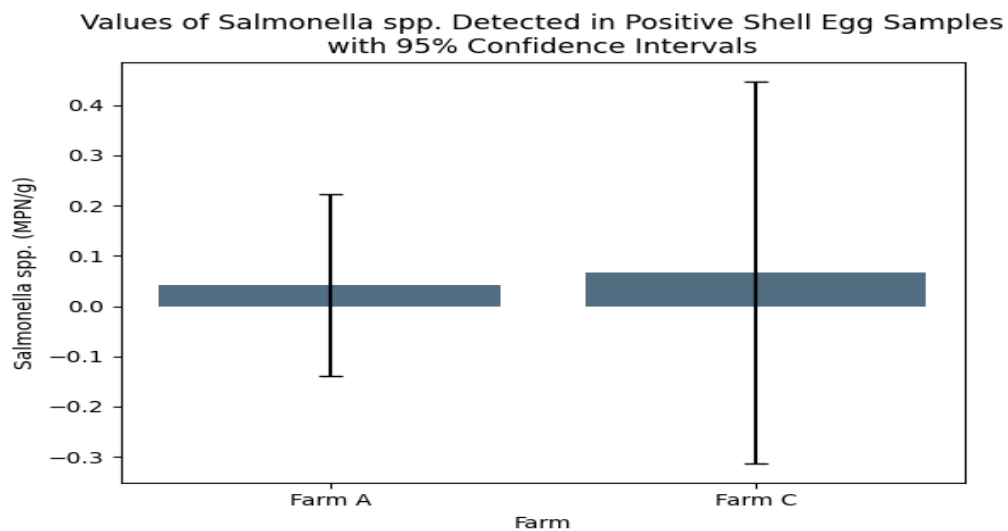


Figure 2. Mean values of *Salmonella* detected in positive shell egg samples from farms A and C, expressed as most probable number (MPN/g). Error bars represent 95% confidence intervals

Compared with international reports, the lower prevalence and bacterial loads observed in Santander may reflect the impact of biosecurity programs, vaccination strategies, and monitoring activities promoted by national authorities and poultry industry organizations. Similar reductions in egg-associated *Salmonella* prevalence have been documented in countries that implemented mandatory control programs

targeting *S. Enteritidis* at the farm level (FAO, 2023). Still, with the numbers of eggs being sent to the market every day, 1.4% prevalence represents a large number of contaminated eggs in the market, involving a large number of consumers potentially being exposed to *Salmonella* on eggs. However, the presence of unregistered or informal egg-producing farms represents a critical gap in the food safety system and may contribute

disproportionately to consumer exposure, as these operations may lack adequate sanitary controls and official oversight.

The findings of this study underscore the importance of maintaining and strengthening microbiological surveillance throughout the egg production and distribution chain. Establishing regional baseline data is

CONCLUSION

This study establishes baseline data on the prevalence and concentration of *Salmonella* in commercial shell eggs produced in Santander, Colombia. The overall prevalence was low (1.39%), and only one isolate was presumptively identified as *Salmonella enterica* serovar Enteritidis (0.27%). Quantitative analysis revealed low bacterial loads, with an average concentration of 0.052 MPN/g among positive samples.

Despite the low prevalence observed, the detection of *Salmonella* spp and *S. Enteritidis* confirms that commercial shell eggs remain

AUTHOR CONTRIBUTIONS

The authors declare no conflict of interest

essential for tracking trends over time, supporting quantitative microbial risk assessment, and evaluating the effectiveness of control measures. Moreover, harmonization with international standards and surveillance frameworks can enhance the competitiveness of the Colombian egg industry while protecting public health.

a potential vehicle for foodborne transmission, particularly when consumed raw or undercooked. These findings highlight the importance of sustained microbiological surveillance, strict biosecurity measures, and effective control programs throughout the egg production chain.

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