Characterization of *Salmonella* from Commercial Egg-Laying Hen Farms in a Central Region of Colombia

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SUMMARY. Salmonellosis affects humans more frequently than any other foodborne disease, and it causes severe economic losses in the poultry industry. A cross-sectional study was carried out to estimate the prevalence of *Salmonella* spp. in laying hen farms in the Tolima region of Colombia. Fifteen egg-laying hen farms were sampled, and a total of 589 samples were cultured to isolate *Salmonella* spp. A total of 14 isolates of *Salmonella* spp. were recovered from five farms, resulting in a prevalence of 33.33% (95%, confidence interval = 14%–53%) at the farm level. *Salmonella* spp. were recovered from eggshells (57.15%, *n* = 8), feed (28.57%, *n* = 4), and environmental samples (14.29%, *n* = 2). Farm practices, such as the milling of feed (odds ratio [OR] = 24) and the storage of eggs in the henhouses (OR = 11.25), in addition to the feed type (OR = 7.64) and the use of bamboo for construction of the facility (OR = 5.24), were identified as risk factors for *Salmonella* spp. The 14 isolates were identified as *Salmonella* Enteritidis (*n* = 6) and *Salmonella* Shannon (*n* = 8), and both serovars were resistant to a number of antibiotics. Pulsed-field gel electrophoresis presented three different *Xba*I macrorestriction patterns. The *Salmonella* Enteritidis isolates all presented a single pattern, whereas the *Salmonella* Shannon isolates were grouped into two distinct patterns. The results indicate that *Salmonella* spp. could be recovered from various sources at laying hen farms, and eggshell contamination is a particular concern.

RESUMEN. Caracterización de *Salmonella* de granjas comerciales de gallinas de postura en una región central de Colombia.

La salmonelosis afecta a los humanos con mayor frecuencia que cualquier otra enfermedad transmitida por alimentos y causa graves pérdidas económicas en la industria avícola. Se llevó a cabo un estudio transversal para estimar la prevalencia de *Salmonella* spp. en explotaciones de gallinas de postura en la región del Tolima en Colombia. Se muestrearon quince granjas de gallinas de postura y un total de 589 muestras fueron cultivadas para aislar *Salmonella* spp. Un total de 14 aislamientos de *Salmonella* spp. fueron recuperados de cinco granjas, resultando en una prevalencia del 33.33% (95%, intervalo de confianza = 14%–53%) a nivel de granja. Se recuperó *Salmonella* spp. a partir de cascarones de huevo (57.15%, *n* = 8), de alimento (28.57%, *n* = 4) y de muestras ambientales (14.29%, *n* = 2). Algunas prácticas, tales como la molienda del alimento (OR = 24), el almacenamiento del huevo en las casetas avícolas (OR = 11.25), el tipo de alimento (OR = 7.64) y el uso del bambú para la construcción de las instalaciones (OR = 5.24), fueron identificados como factores de riesgo para *Salmonella* spp. Las 14 cepas fueron identificadas como *Salmonella* Enteritidis (*n* = 6) y *Salmonella* Shannon (n = 8) y ambos serotipos fueron resistentes a varios antibióticos. La electroforesis en gel de campo con pulsaciones mostró tres patrones diferentes de macrorrestricción por la enzima *Xba*I. Todos los aislamientos de *Salmonella* Enteritidis mostraron un patrón único, mientras que los aislamientos de *Salmonella* Shannon se agruparon en dos patrones distintos. Los resultados indican que la *Salmonella* spp. pudo ser recuperada de varias fuentes en las granjas de gallinas de postura y la contaminación del cascarón es de especial preocupación.

Key words: *Salmonella*, laying hen farm, eggs, foodborne diseases, risk factors

Abbreviations: BPW = buffered peptone water; ICA = Instituto Colombiano Agropecuario; OR = odds ratio; PFGE = pulsed-field gel electrophoresis

Salmonellosis is one of the most important zoonotic diseases throughout the world; the infection causes significant morbidity and mortality in both humans and animals as well as considerable economic losses in the avian industry (7,40,44). In the United States, it is estimated that 9.4 million cases of foodborne illnesses occur every year, and nontyphoidal *Salmonella* may be responsible for approximately 11% of these cases (1 million), corresponding to 19,586 hospitalizations and 378 deaths (42). Subspecies of *Salmonella enterica* are transmitted by eggs, egg-containing foods, and inadequately cooked poultry. The disease in humans is characterized by gastroenteritis, enteric fever (typhoid and paratyphoid), bacteremia, localized infection, and an asymptomatic chronic carrier stage (7,51). From 1997 to 2011, the National Institute of Health of Colombia (26) reported 5641 cases of acute diarrheal disease caused by *Salmonella enterica*, mostly transmitted by poultry products. Of these, *Salmonella* Typhimurium and *Salmonella* Enteritidis are the most prevalent serovars, followed by *Salmonella* Typhi 6 (12,13,26).

Salmonellosis in poultry is commonly caused by *Salmonella* Pullorum and *Salmonella* Gallinarum, the pathogenic serovars responsible for pullorum disease and fowl typhoid, respectively (17,45). Outbreaks of salmonellosis may also be caused by the subspecies *Salmonella arizonae* (10) and by the vaccine strain, *Salmonella* Gallinarum (53). The disease is characterized by diarrhea, anorexia, and dehydration in both young and adult birds, resulting in high mortality (36,40). However, in most cases, the flocks are coinfected with serotypes that do not have a significant impact on avian health but that do cause severe disease in humans, such as *Salmonella* Enteritidis (40). Therefore, the consumption of poultry products, including eggs, has been associated with numerous cases of human salmonellosis (3,4,30,33).

Colombia produces approximately 11,000 million eggs per year, and an estimated 236 eggs are consumed per person each year in

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Colombia (16). Governmental and academic institutions, in addition to the poultry and egg industry, are currently assessing the health status of the flocks in Colombia (active surveillance) and evaluating the quality of poultry products by conducting microbiologic tests to determine the potential impact on public health. Although both active and passive surveillance systems are insufficient for monitoring many diseases in poultry and other meat production systems in Colombia, individual efforts have identified a variety of *Salmonella* subtypes in poultry that have contributed to the epidemiology of this disease in this country (14,15,40,48). In addition, these studies have identified a variety of antibiotic resistance patterns in *Salmonella* and emphasized the need for better surveillance systems in commercial laying hen farms.

The Tolima region is a major poultry producer. However, epidemiologic studies on the health status of the poultry and the safety of the products are very limited, and only fragmented data are available. To investigate the potential impact of *Salmonella* in this region, a preliminary cross-sectional study was conducted to establish the prevalence, molecular characteristics, and potential risk factors for *Salmonella* contamination at laying hen farms.

MATERIALS AND METHODS

Study population. The Tolima district is located between the central and eastern mountains of the Colombian Andes; it has 42 municipalities with approximately 2 million laying hens at 45 laying hen farms (25). Together with the Cundinamarca, Huila, and Boyacá districts, Tolima represents the largest egg producer in Colombia.

Study and survey design. A cross-sectional study was designed to estimate the prevalence of *Salmonella* spp. in a sample of 15 out of 45 egg-laying hen farms over a 6-mo period from January to June 2013. The farmhouses were registered with biosecurity protocols established by the Instituto Colombiano Agropecuario (ICA) and were in the egg-production period during the course of the study. The sampled farms were selected by convenience. We chose a nonprobabilistic method because the study depended on the farm owner's decision to participate in the study (farm owners frequently prohibit access to their facilities for biosecurity reasons or because they are concerned about the potential findings). Thus, only 15 commercial egg-laying farms allowed us to conduct confidential sampling. Each farm was sampled only once, and sampling at each site was performed during different weeks to reduce the risk of spreading pathogens from farm to farm. Two categories were established to discriminate the larger and smaller laying farms: type 1 farms (holding >5000 hens) and type 2 farms (holding <4999 hens). In type 1 farms (eight), three flocks (henhouse) were sampled, whereas in type 2 farms (seven), only one flock was sampled, for a total 31 sampled flocks.

Sample collection. A total 31 flocks were sampled from 15 farms, and 589 samples were processed for *Salmonella* spp. isolation. The samples included cloacal samples ($n = 310$), samples of feed ($n = 31$) and water ($n = 31$), boot-swab samples ($n = 31$), egg samples ($n = 155$), and fecal samples provided by the henhouse operators ($n = 31$). The cloacal samples were prepared by pooling five individual cloacal swabs (10 samples/flock). The feed samples consisted of approximately 250 g of feed collected in sterile bags (Nasco®, Fort Atkinson, WI), and the water samples consisted of 250 ml of water collected in sterile bags (Nasco). The boot-swab samples were obtained by covering plastic boots with cotton socks, which were collected after walking the length of the henhouse. The egg samples were prepared by pooling five eggs (25 eggs/flock). The fecal samples were obtained from a fecal swab. Although the total number of samples was 589, the study included 1550 birds and 775 eggs. The samples were placed on frozen gel packs for transport, and they were processed at the Laboratory of Veterinary Diagnosis, University of Tolima. The transport time was less than 3 hr before processing.

Salmonella isolation and identification. Microbiologic culture for the isolation of *Salmonella* spp. followed specific standard procedures, such as the ISO/6579: 2002/AMD1: 2007, the WHO Isolation of *Salmonella* spp. Laboratory Protocol, the Colombian Technical Standard 4574, the standard issued by the ICA, and the standard protocols issued by the National Institute of Food and Drug Monitoring (27). Briefly, each sample was pre-enriched in buffered peptone water (BPW) as follows: 25 g of feed and 25 ml of drinking water were separately mixed with 225 ml of BPW (Oxoid, Basingstoke, Hampshire, United Kingdom); the pooled cloacal swabs and boot swabs were mixed with 25 ml of BPW; pools containing the shells of five eggs were placed in a beaker, macerated, and mixed with 250 ml of BPW; the eggs' contents were homogenized in a stomacher bag for 1 min and mixed with BPW at a 1:1 ratio. All samples were incubated at 37 ± 1 C for 24 hr. Then, 0.1 ml of pre-enrichment medium was inoculated into 10 ml of Rappaport Vassiliadis broth and incubated at 41.5 ± 1.0 C for 24 hr. The fecal samples from the staff were directly inoculated in Rappaport Vassiliadis and tetrathionate broth and incubated at 41.5 ± 1.0 C for 24 hr. A second aliquot (0.1 ml) of pre-enriched medium was inoculated into 10 ml tetrathionate broth and incubated at 37 ± 1 °C for 24 hr. Next, an aliquot of Rappaport Vassiliadis and tetrathionate cultures were inoculated into xylose lysine deoxycholate and MacConkey selective solid media and incubated at 37 ± 1 C for 24–48 hr. Suspect *Salmonella* colonies were confirmed by culture in xylose lysine tergitol-4 agar at 35 ± 1 C for 18–24 hr and Rambach agar at 37 C for 24–48 h. Subsequently, the *Salmonella* colonies were tested for agglutination with Poly A-I & Vi antiserum (Difco® 222641; Becton Dickinson and Co, Sparks, MD). Finally, the *Salmonella* isolates were biochemically characterized using the API-20E® (BioMérieux, Marcy l'Etoile, France) enteric identification system. Farms that were positive for *Salmonella* were identified when at least one sample gave positive results by bacteriologic culture.

Salmonella serotyping. *Salmonella* isolates were serotyped following the Kauffman-White scheme (5) for O and H antigens and by using commercial antisera (Difco, Becton Dickinson and Co). The serotypes of *Salmonella* are based on the nomenclature described by the Judicial Commission of the International Committee on Systematics of Prokaryotes (28). The tests were carried out at the National Laboratory of Veterinary Diagnosis of ICA (Bogotá, Colombia).

Antibiotic susceptibility and resistance. *Salmonella* isolates were subjected to an antimicrobial disk diffusion susceptibility test following the Kirby-Bauer method using the BD Phoenix™ PMIC/ID panels (Becton Dickinson) and the categories established by the Clinical and Laboratory Standards Institute (9).

Epidemiologic variables and analysis. The farm manager or veterinarian in charge of the facility was interviewed with a questionnaire before sampling at each farm. The questionnaire included basic farm-level information, including questions about the farm location, the distance to population centers, the number of sheds and the total capacity, the age of the birds, the genetic line of the birds, the vaccination status, the structure and the type of ventilation system, the number of pens, the production capacity, the pest control program (rodents, flies and others), the source and pH of the water, the disinfection protocols, feed manufacturing practices, raw materials, biosecurity practices and staffing, personnel training, and the health condition of the operators. All information was collected and, when possible, verified visually by the researcher at the time of sampling. The researcher also confirmed that the sampled farm had clinically healthy animals. The questionnaire was prepared based on a survey protocol (Deed 1183) issued by the authority (ICA) and included 103 questions. This instrument was tested at two farms prior to being used in the study. All farm-level, microbiologic, and serotyping data were compiled and analyzed using Epi-Info® software (Epi Info 7, Centers for Disease Control and Prevention, Atlanta, GA). The prevalence of *Salmonella* spp. at the laying hen farms was calculated by dividing the number of farms classified as positive for *Salmonella* by the total number of sampled farms. The epidemiologic variables associated with the presence of *Salmonella* spp. were analyzed by ANOVA. The chi-square test or

Fisher's exact test was used to evaluate the associations between categoric variables and positivity to *Salmonella*.

Molecular characterization by pulsed-field gel electrophoresis (PFGE). PFGE was performed using a CHEF-DR III system (Bio-Rad Laboratories, Hercules, CA) at the Laboratory for Antimicrobial Resistance of ICA, following the recommended PulseNet protocol (8,41). Briefly, the genomic DNA from each *Salmonella* isolate was released into agarose plugs and in-gel digested with the restriction enzyme *Xba*I (Promega, Madison, WI). The DNA fragments were separated on a PFGE-certified 1% agarose gel (Bio-Rad) with $0.5\times$ Tris-borate-ethylenediaminetetraacetic acid running buffer for 17 hr at 6 V/cm with increasing pulse times. *Salmonella* Braenderup H9812 DNA digested with *Xba*I was used as a reference and size standard. The gels were stained with ethidium bromide and analyzed using the Gel Compare II® software (Applied Maths, Sint-Martens-Latem, Belgium). The similarity was calculated by the Dice coefficient, and a dendrogram was constructed by cluster analysis using the unweighted pair group method with arithmetic mean. A band position tolerance of 1.5% was used for analyzing the PFGE fingerprints.

RESULTS

***Salmonella* spp. in commercial laying hen farms in Tolima.** Five out of 15 farms were positive for *Salmonella* spp.; thus, the prevalence at the farm level was 33.33% (confidence interval = 14%–53%) with a 95% confidence level. The positive farms represented both type 1 ($n = 4$) and type 2 ($n = 1$) farms, and *Salmonella* was isolated more frequently from type 1 farms (>5000 birds) than from type 2 farms. Of the 589 analyzed samples, *Salmonella* was isolated most frequently from the egg surface (57.15%, $n = 8$), followed by the feed samples (28.57%, $n = 4$) and environmental (14.29%, $n = 2$) samples, whereas the cloacal swabs were all negative. The farms that were positive for *Salmonella* included on-floor (80%) and on-cage (20%) farmhouses, with an on-floor bird density ranging from 7 to 10 hens/m². Approximately 80% of the on-floor farmhouses also had facilities that were constructed from bamboo, and 60% of those farms packaged their eggs at the sampled site. In addition, 80% of the farms had a small river as the water supply, and only 20% used water supplied by a company. The age of the sampled hens ranged from 18 to 96 wk (laying period).

Serotypes of *Salmonella*. The *Salmonella* isolates belonged to serogroups D and E, and 57.15% ($n = 8$) of *Salmonella* serovars were identified as *Salmonella* Shannon whereas 42.85% ($n = 6$) were *Salmonella* Enteritidis. *Salmonella* Shannon was only isolated from type 1 farms, whereas *Salmonella* Enteritidis was isolated from both types of farms.

Antibiotic susceptibility and resistance of *Salmonella* isolates. The 14 isolates of *Salmonella* spp. were resistant to amikacin, cephalothin, cefoxitin, cefuroxime, and gentamicin; of those isolates, five out of six *Salmonella* Enteritidis isolates presented intermediate resistance to nitrofurantoin. Two isolates of *Salmonella* Shannon were resistant to trimethoprim-sulfamethoxazole, and all isolates were sensitive to amoxicillin clavulanate, ampicillin, aztreonam, cefepime, ceftazidime, ceftriaxone, ciprofloxacin, imipenem, levofloxacin, meropenem, piperacillin-tazobactam, and tigecycline.

Risk factors for *Salmonella* at laying hen farms in the Tolima district. The epidemiologic data from each laying hen farm were categorized in to six main groups that included 1) general farm information, 2) animal resources, 3) *Salmonella* prevention measures, 4) facilities and equipment, 5) water quality and biosecurity measures, and 6) health condition of flock operators. From those categories, the general farm information, and particularly variables such as feed milling performed by the farmer (on-site feed mill) at the farmhouse, eggs stored in the henhouses, henhouses with a soil floor, feed presentation (mash vs. pellet), lack of a *Salmonella* diagnosis and surveillance program, and type of facility structure were found to be associated ($P \leq 0.05$) with the presence of *Salmonella* spp. using univariate logistic regression analysis. The most important variables associated with *Salmonella* were on-site feed milling (odds ratio [OR] = 24), egg storage in the henhouses (OR = 11), henhouses with a soil floor (OR = 7.88), presentation of feed as a mash (OR = 7.64), lack of a *Salmonella* diagnosis and surveillance program (OR = 3.17), and a bamboo facility structure (OR = 5.24; Table 1).

PFGE analysis. The *Xba*I-digested genomic DNA from all *Salmonella* isolates resulted in 11–12 bands that were grouped into three main macrorestriction patterns by PFGE with similarity coefficients between 84% and 100%. The gels were analyzed by the Gel Compare II software, and a dendrogram is shown in Fig. 1. This analysis revealed three distinct clusters corresponding to the two identified serovars; the first and the second clusters included all *Salmonella* Shannon (8/14) isolates with a similarity coefficient of 80.4% (Fig. 1). The first cluster was characterized by a distinctive second band with a small size in two (UTSS13005 and UTSS13007) of the *Salmonella* isolates compared to the second cluster of *Salmonella* Shannon. The third cluster corresponds to all *Salmonella* Enteritidis, which showed a 100% similarity coefficient.

DISCUSSION

This study was conducted to establish the prevalence of *Salmonella* spp. at commercial laying hen farms in the Tolima district, a region of Colombia with high egg production but limited information on the health status of poultry. Using standard microbiologic culturing techniques for *Salmonella* isolation followed by serotyping, antibiotic susceptibility testing, and PFGE typing, we determined the prevalence, serotypes, and antibiotic resistance patterns of the *Salmonella* serovars that circulated from January to June 2013. The data provide an important record for further epidemiologic studies and highlight the need to improve the on-farm biosecurity measures and quality control of raw materials, feed, and poultry products.

The prevalence of *Salmonella* spp. at the farm level was 33.33% (5/15), and the bacterium was isolated from farms with >5000 hens (type 1) and from farms with <4999 hens (type 2), although the majority of *Salmonella* isolates were obtained from type 1 ($n = 4$) farms holding >5000 birds, suggesting that *Salmonella* contamination may increase at large farms. Indeed, a large flock size is a well-known risk factor for *Salmonella* in laying hens in other countries (34). It should be noted that although large farms in the Tolima region hold up to 400,000 birds, the number of workers may remain the same as that at smaller farms, and this might be associated with reduced quality. The prevalence of *Salmonella* obtained in this study was comparable to that reported in commercial laying hen (44.44%) farms in the Antioquia district (48). Taken together, the results strengthen the need for proper diagnosis and surveillance of this pathogen in Colombia's poultry industry. The prevalence of *Salmonella* at laying hen farms in Colombia may be high when compared to other regions of the world. For example, *Salmonella* spp. contamination in laying hens ranges from 17.9% (24) and 18% (2) in France and the United Kingdom, respectively, and a prevalence of 7.1% was reported for *S.* Enteritidis in the United States (19). Meanwhile, higher prevalence levels (60%) have been reported in other regions of Latin America (47). However, the conditions and characteristics of the poultry industry between countries may differ significantly, and any extrapolation may not be completely valid.

Table 1.   Risk factors for *Salmonella* contamination of laying hen farms in the Tolima district, Colombia.[A]

Variables	OR	95% Confidence interval	*P* value
Independent feed mill	24.00	(2.4964–23230.7343)	0.00010
Egg stored in henhouses	11.25	(2.0349–62.1969)	0.026
Henhouse with soil floor	7.88	(1.5596–39.7642)	0.0088
Feed presentation (mash)	7.64	(1.11–52.47)	0.05
Salmonella diagnosis and surveillance	3.17	(1.6336–6.1383)	0.00017
Facility structure in bamboo	5.24	(1.0567–25.966)	0.035

[A]Data for 31 henhouses.

Salmonella may originally contaminate the eggs during their development within the ovary or during their passage through the oviduct, and horizontal transmission may occur through transshell contamination (23,33,35). The transovarian route is considered the main route for the contamination of eggs by *Salmonella* Enteritidis, eventually leading to human infection upon consumption (4). Of note, among the 14 *Salmonella* isolates in this study, none was recovered from cloacal swabs, but eight were isolated from the egg surface (57.15%), four were isolated from feed samples (28.57%), and two were isolated from environmental (14.29%) samples. The lack of *Salmonella* isolated from cloacal swabs may be because of reasons that have been described previously (22). Increasing the number of swabs in the pool may increase the ability to detect *Salmonella* (1), and introducing the cloacal swabs directly into peptone water may also increase the detection level (6); this methodology should be considered in further studies to more accurately estimate the level of infection. In addition, environmental sampling has been found to be more sensitive than bird sampling (22). Nevertheless, the results of our study are similar to those reported for commercial laying hen farms in Spain, where the proportion of *Salmonella* isolated from egg shells (34%) was higher than that from cloacal swabs (4%) (20), and in the Parana State of Brazil, where *Salmonella* was isolated only from the discarded hatching eggs of broiler hens (prevalence of 52%) that are intended for human consumption (31). Together, the results reinforce the fact that the prevalence of *Salmonella* is variable between flocks, as is the efficacy of cleaning and disinfection procedures, indicating the need for regular and sensitive monitoring of flocks for *Salmonella* (55).

A number of potential risk factors for *Salmonella* contamination on laying hen farms in the Tolima district were identified in this study. The risk associated with on-floor farming (OR = 7.8) probably indicates insufficient floor cleaning and disinfection, deficient sanitization of the hens' recycled bedding, and persistent environmental contamination from previous laying hen flocks that were positive for *Salmonella* (24,49,52), a predominant problem on commercial laying farms (21). On the other hand, the cage system is associated with the lowest risk of infection by *Salmonella* Enteritidis (34); however, other researchers have reported contrasting results in other countries (32,35,46), including the absence of such differences (38). Many laying hen farms in the Tolima region use a soil floor for economic reasons, thus it is necessary to evaluate the cost-benefit of using more appropriate facilities for commercial laying hen farms in this region.
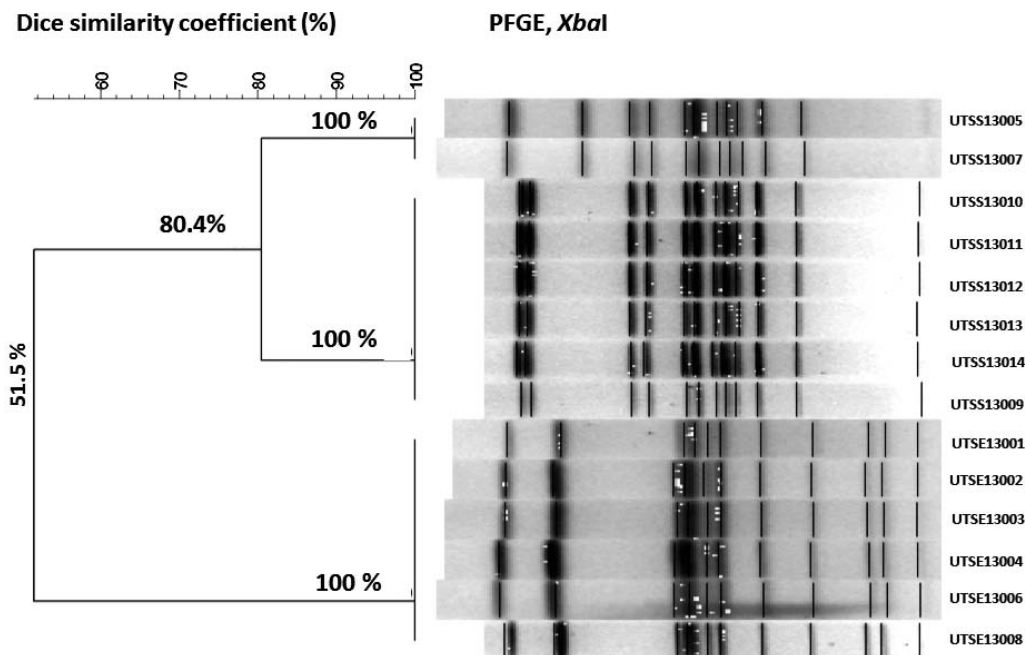

Fig. 1.   Macrorestriction patterns of 14 *Salmonella* Shannon and *Salmonella* Enteritidis serovars generated by PFGE of *Xba*I-digested genomic DNA. A similarity analysis was performed using the Dice coefficient, and the dendrogram was generated by the unweighted pair group method with arithmetic averages using the Gel Compare II® software (Applied Maths). Lanes 1 to 14 (UTS13001–UTS13014) correspond to the genomic DNA from each *Salmonella* isolate from the laying hen farms in the Tolima region.

Another risk factor was the use of bamboo in the structure of the facilities (OR = 5.2). Bamboo is a natural material that may represent a reservoir of infection with limited options for disinfection. Finally, 60% of the positive farms stored the eggs in the henhouses, and 80% used a small river as the water supply, two critical variables that may favor *Salmonella* contamination. Storing eggs inside the henhouse (OR = 11.25) without temperature control may favor egg contamination by *Salmonella*. Temperature fluctuations from 18 C at night to 37 C or more during the day may allow *Salmonella* contamination to reach a level that can cause human disease (18). It was estimated that eggs held at approximately 18 C have 25-fold higher risk of testing positive for *Salmonella* than eggs held at temperatures of 7.2 C (39). Thus, the regional poultry industry should focus on providing appropriate conditions for egg storage and transportation.

A main risk factor for *Salmonella* contamination at the laying hen farms in this study was on-farm feed milling (OR = 24). This risk factor has been identified in other studies demonstrating that contaminated raw material and feed ingredients were the main source of *Salmonella* contamination during the feed milling process (11,29,37,43). In Spain, Torres reported a *Salmonella* prevalence of 28% in feed mills, although the proportion of feed mills contaminated with *Salmonella* serovars relevant to public health was only 2.7% (50). In this study, *Salmonella* Shannon, but not *Salmonella* Enteritis, was isolated from feed, thus the relevance and potential impact of this serovar remains to be investigated. The feed ingredients were also tested for *Salmonella*, and the bacteria were detected in several raw materials, including soybean meal, and the molecular identification of those isolates is in progress. In addition, the feed presentation as a mash (OR = 7.6) was also found to be an important risk factor when compared to pelleted feed. Previous studies have suggested that the pelleting process itself can reduce the isolation of *Salmonella* from the feed by approximately 50% (29,50).

Regarding antibiotic resistance, both *Salmonella* serovars showed similar patterns of resistance and susceptibility to a panel of antibiotics. However, the two *Salmonella* Shannon (UTSS13005 and UTSS13007) isolates that were characterized by a second small band in the *Xba*I digest (Fig. 1) and clustered separately from the other *Salmonella* Shannon isolates were the only isolates resistant to trimethoprim-sulfamethoxazole, suggesting a link between this particular phenotype and the genotype responsible for the low coefficient of similarity with the other *Salmonella* Shannon isolates. It would be interesting to know whether this resistance pattern evolved spontaneously or is simply the result of a selective pressure related to the use of this antibiotic to control pathogenic strains of *Salmonella*. Several *Salmonella* Enteritidis isolates from poultry have been found to be resistant to trimethoprim-sulfamethoxazole (54); however, the relevance to *Salmonella* Shannon is currently unknown. The coefficient of similarity between the *Salmonella* Enteritidis isolates indicate that only one clonal group, which was characterized by a similar *Xba*I PFGE pattern, circulated in the laying hen farms in the Tolima region during the course of this study.

CONCLUSIONS

Salmonella Shannon and *Salmonella* Enteritidis were isolated from environmental samples, feed, and eggshells at laying hen farms in the Tolima district, Colombia, during the period January–June 2013. This study indicates that the poor management conditions, deficiencies in the cleaning and disinfection procedures, the lack of biosecurity measures, and the low educational background of the operators may contribute to *Salmonella* contamination of the egg

surface and present a potential risk to consumers. This study represents the first report of *Salmonella* Shannon in Colombia and reveals the potential effects of antibiotic pressure on this microorganism as well as the need for proper diagnosis and surveillance systems for *Salmonella* Enteritidis in poultry products. The identified risk factors may facilitate the design of novel strategies to improve biosecurity measures and control this microorganism during primary production. Additional epidemiologic investigations are necessary to estimate the potential impact of *Salmonella* on the poultry industry and public health.

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