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SCIENTIFIC ARTICLE
Animal Epidemiology

Association between non-typhoidal *Salmonella* isolated from commercial poultry sheds and associated factors in Paraná, Brazil: Cross-sectional retrospective study

Nayara Dias da Silva[1,*] https://orcid.org/0000-0002-1943-4779
Ellen Elizabeth Laurindo[2] https://orcid.org/0000-0003-1255-1347
Camila Marinelli Martins[3,4] https://orcid.org/0000-0002-8425-5769
Ricardo Michael Pinheiro Silveira[5] https://orcid.org/0000-0002-5664-7079
Claudinei Taborda da Silveira[5] https://orcid.org/0000-0001-6289-6306
Elizabeth Santin[1] https://orcid.org/0000-0003-3110-0875

1. Universidade Federal do Paraná – Câmpus de Ciências Agrárias – Departamento de Medicina Veterinária – Curitiba (PR), Brazil.
2. Ministério da Agricultura, Pecuária e Abastecimento – Departamento de Promoção Comercial e Investimentos – Brasília (DF), Brazil.
3. Universidade Estadual de Ponta Grossa – Câmpus Uvaranas – Departamento de Enfermagem e Saúde Pública – Ponta Grossa (PR), Brazil.
4. AAC&T Consultoria em Pesquisa – Diretoria de Pesquisa – Curitiba (PR), Brazil.
5. Universidade Federal do Paraná – Câmpus Centro Politécnico – Departamento de Geografia – Curitiba (PR), Brazil.

*Corresponding author: sdiasnay@gmail.com

ABSTRACT

The epidemiology of salmonellosis in poultry is complex, which makes it difficult to identify the origin and spread of this disease in poultry farms. The aims of this study were to characterize the spatial distribution of *Salmonella enterica* in epidemiological units in Paraná, Brazil; and to investigate correlations between this microorganism and associated factors. Among the epidemiological units, 78 of 243 (32.10%) were positive. Spatially, the northwestern and western regions had higher concentrations of positive cases than the other regions. In bivariate analyses, the presence of other animal species in the epidemiological unit (prevalence ratio, PR = 0.64; 95% confidence interval, CI = 0.43–0.95; p = 0.022) and proximity to establishments at risk (PR = 0.51; 95% CI = 0.32–0.81; p = 0.001) did not influence positivity, but the average population per poultry shed (between 30,501 and 32,500; PR = 2.57; 95% CI = 1.72–3.83; p = 0.001) was associated with *Salmonella* positivity. Multiple logistic regression demonstrated that the average population per poultry shed, presence of surrounding risk-posing establishments and presence of surrounding poultry sheds produced a significant multiple model for *S. enterica*. The results indicated that the presence of *S. enterica* may be related to higher density broiler in poultry sheds, presence of surrounding poultry sheds, proximity between positive and negative epidemiological units and altitude of the municipality. The information obtained showed that some factors were related to positivity for this microorganism and emphasizes the importance of serotyping to obtain other epidemiological data.

Keywords: geographical distribution; density; distance; altitude; serovars, legislation.

INTRODUCTION

Brazil is prominent within the poultry industry, as the second largest producer and the largest exporter of chicken meat in the world. In 2019, the total production of this protein was 13,245 million tonnes and 32% of this quantity were exported. The Brazilian poultry industry is concentrated in the southern states of the country: Paraná is the largest producer (34.69%), followed by Santa Catarina (15.40%) and Rio Grande do Sul (14.32%). In 2019, Paraná was also responsible for 39.13% of Brazilian exports (ABPA, 2020). To ensure food safety and quality, continuous improvement in

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all sectors of the production chain is required, including poultry farm management, nutrition and biosecurity, and control over important pathogens within poultry and public health, such as *Salmonella* (PULIDO-LANDÍNEZ, 2019).

The genus *Salmonella* belongs to the family Enterobacteriaceae and comprises the species *Salmonella enterica* and *Salmonella bongori* (BRENNER et al., 2000), which have more than 2,600 known serotypes (EFSA; ECDC, 2014). Most of the pathogenic serovars of *Salmonella* spp. belong to the species *S. enterica*. They are commonly named based on the geographical location or the animal species from which they were isolated. *Salmonella* serotyping is important because it allows the identification of more pathogenic serovars and assessment of epidemiological factors that may assist in controlling these agents in poultry production (GRIMONT; WEILL, 2007). Two of these serovars are bird specific and are responsible for causing pullorum disease (*S. enterica* subsp. *enterica* serovar Pullorum) and fowl typhoid (*S. enterica* subsp. *enterica* serovar Gallinarum), which lead to occurrences of diarrhea and septicemia and high mortality rates (BARROW; FREITAS NETO, 2011). Nontyphoidal *Salmonella* strains are represented by any serotype of the genus, except *S.* Pullorum and *S.* Gallinarum. Most nontyphoidal *Salmonella* serovars do not significantly affect the zootechnical performance of poultry because they do not cause any symptoms in the host (GAST; BENSON, 1995). However, they are able to colonize the intestine and can reach the bloodstream. Thus, they can be identified in other organs such as the spleen, liver and ovaries (HUMPHREY, 2004; SHIVAPRASAD et al., 1990). Nontyphoidal *Salmonella* strains are able to colonize the intestinal tract, contaminate chicken carcasses at the time of slaughter and cause harm to consumer health (VOSS-RECH et al., 2015).

In 1994, an official program for controlling *Salmonella* in Brazilian poultry was implemented through Ordinance 193 of the Ministério da Agricultura, Pecuária e Abastecimento (MAPA) (BRAZIL, 1994). This ordinance constituted the Programa Nacional de Sanidade Avícola (PNSA), with the objective of establishing control and eradicate methodologies for the main diseases that affect poultry, including salmonellosis. The nontyphoidal *Salmonella* serotypes involved in this program were *S. enterica* subsp. *enterica* serovar Enteritidis and *S. enterica* subsp. *enterica* serovar Typhimurium (BRAZIL, 2003): these are pathogens of great importance for public health worldwide. Other regulations relating to *Salmonella* monitoring have included Normative Instructions No. 10 of April 11, 2013 (BRAZIL, 2013), and No. 20 of October 21, 2016 (BRAZIL, 2016), which were established by MAPA and refer to epidemiological surveillance within the poultry production. Given the complex epidemiology of *Salmonella*, other important serovars, such as Minnesota, Infantis, Heidelberg, Senftenberg and Mbandaka, have been isolated from poultry shed samples in Brazil (VOSS-RECH et al., 2015). Another study conducted in the state of Paraná showed that among 342 swab specimens processed, *S.* Heidelberg was the one most frequently isolated (12.82%), followed by *S.* Mbandaka and *S.* Newport, which accounted for 10.25%. *Salmonella* Schwarzengrund, Enteritidis and Orion each presented frequencies of 7.70% (PANDINI et al., 2015). Preliminary data from 2018 from the Centers for Disease Control and Prevention (CDC) Foodborne Diseases Active Surveillance Network (FoodNet) showed that, among the cases of foodborne illnesses, *Salmonella* had the second highest incidence (18.3 cases per 100,000 population), which was only behind *Campylobacter* (19.5). Among the 7,013 (87%) serotyped *Salmonella* isolates, the three most common were Enteritidis (2.6 per 100,000 population), Newport (1.6) and Typhimurium (1.5) (TACK et al., 2019). In the European Union in 2017, 91,662 human salmonellosis cases were confirmed and the reporting rate for human salmonellosis was 19.7 cases per 100,000 inhabitants. The most frequent serovars were *S.* Enteritidis, *S.* Typhimurium and monophasic *S.* Typhimurium. Food and animal data showed that *S.* Enteritidis was associated mainly with laying hens and chicken meat (EFSA; ECDC, 2018).

Bacteria of the genus *Salmonella* are widely distributed in nature and can affect different reservoirs. They are extremely resistant and can survive in different types of environments. These characteristics make them easily propagated and difficult to control (EFSA; ECDC, 2014). *Salmonella* is commonly found in production animals, in the intestines of pigs, poultry and cattle, but can also be present in domestic animals such as dogs, cats, birds and reptiles. The isolation of this microorganism in animal production facilities is common and, therefore, it is also commonly present in foods of animal origin (DERACHE et al., 2009). One of the main risk factors associated with *S. enterica* infection in poultry farms is animal density (ELGROUD et al., 2009), since the excretion of bacteria can infect the whole batch and even nearby batches without apparent clinical signs (PEREIRA et al., 1999). The presence of other animals in addition to poultry may also represent a risk factor for *S. enterica* infection in broilers (ELGROUD et al., 2009), since people transiting between breeding establishments can carry the bacteria, thus favoring agent dissemination (LE BOUQUIN et al., 2010).

A study in Brazil showed that combination of caloric stress and infection with presence of *Salmonella* Enteritidis can disturb the intestinal barrier of poultry, promote migration of the bacteria to other organs and cause intestinal inflammation, thus compromising productive performance (QUINTEIRO-FILHO et al., 2012). Hence, environmental factors such as temperature increase caused by changes to the weather and type of poultry housing used can alter immune system functioning and decrease the resistance to infection, thereby impairing poultry performance (QUINTEIRO-FILHO et al., 2010).

The reduction of the prevalence of *Salmonella* in poultry production requires detailed knowledge of the risk factors in the poultry production system (MARÍN et al., 2011). Considering the importance of salmonellosis in poultry farming and

within public health, information relating to the epidemiology of these bacteria is important for understanding the evolution of these microorganisms and other particularities, and, thus, for enabling the development of new control methodologies. In this context, this study aimed to characterize the epidemiological profile of nontyphoidal *Salmonella* isolates in broilers in the state of Paraná, southern Brazil.

MATERIALS AND METHODS

Data collection

Data were provided by the Official Veterinary Service (OVS) of the state of Paraná, based on information/reports from visits and collections conducted in unregistered epidemiological units with low biosafety, which are present in the region studied. Unregistered epidemiological units are those that do not meet the minimum biosafety requirements established through Ordinance No. 290 of November 9, 2017 of the Animal Health Protection System of the Agência de Defesa Agropecuária do Paraná (ADAPAR) (PARANÁ, 2017) and Normative Instruction No. 56 of December 4, 2007 of MAPA of Brazil (BRAZIL, 2007). These requirements include management, location and isolation of facilities; physical and natural barriers; access and traffic flow control; water and feed care; poultry health program; personal training plan; contingency plan; and pest control program, among other factors. The OVS does not have data available on registered epidemiological units. When samples were collected, only unregistered epidemiological units were evaluated.

By definition, an epidemiological unit is a physical unit of poultry production, composed of one or more poultry sheds that house a group of birds of the same species and age. These units are under common production management and must be isolated from other poultry production activities through natural or artificial physical barriers and must not have structures and activities that are outside the production process, such as homes, vehicles, plantations or other creations within them. In laying hen establishments, concomitant presence of birds of the same species at different ages is permitted.

Sample collection for *Salmonella* analysis

All the parameters/procedures used in visits were in compliance with Normative Instructions No. 56 of December 4, 2007 (BRAZIL, 2007), No. 10 of April 11, 2013 (BRAZIL, 2013) and No. 20 of October 21, 2016 (BRAZIL, 2016) from MAPA, Brazil. Most of the data provided did not include serotyping of *Salmonella*; therefore, this study opted to analyze only the presence or absence of this microorganism without considering the serovars. This study on *S. enterica* in broilers in Paraná (visit and collection) was developed between January 2017 and May 2018, in the municipalities where the epidemiological units were located. In total, 243 unregistered epidemiological units were analyzed. This study focused on broilers.

The management sample collection procedure was conducted under the responsibility of the veterinarian of the poultry establishment, and appropriate biosecurity practices were adopted. The samples were sent to accredited laboratories. The samples each comprised four drag swabs, divided into two pools containing two drag swabs that were moistened with conservation medium. It was envisaged that these would be used to sample up to 50% of the surface of the shed chicken. The person responsible for sample collection walked the entire length of the poultry shed with the drag swabs. These comprised sterile disposable socks moistened with conservation medium. This methodology was in accordance with Normative Instruction No. 20 of October 21, 2016, of the Ministry of Agriculture, Livestock and Supply (BRAZIL, 2016).

After collection, the samples were packaged and sent as soon as possible to the laboratory. The humidity was maintained and the temperature was kept between 2 and 8 °C, with a variation of 1 °C upwards or downwards. The samples were sent to the laboratory bearing tamper-proof numbered seals, with a collection form containing identification information relating to the poultry establishment. At the time of sample collection, the birds needed to be free from any effect of antimicrobials for gram-negative bacteria, and free from any influence from products with antimicrobial action in the environment (BRAZIL, 2016).

In establishments that had one to three poultry sheds, all of them were monitored. In those with four poultry sheds, three were monitored. In those with five to ten poultry sheds, four were monitored; and in those with over 11 poultry sheds, five were monitored. The legislation stipulates that samples should be collected as close as possible to the date of poultry batch slaughter, while allowing the results to be known before slaughter. In addition, in poultry sheds that are to be sampled, those with birds that present any clinical signs, those with low zootechnical index and those with birds subjected to situations of stress periods, among other factors that favor pathogen detection, should be prioritized. It also stipulates

that random collections may be performed at any time, and that the number and type of samples to be collected and the number of sheds to be sampled can be increased, based on epidemiological investigations (BRAZIL, 2016).

Salmonella isolation, identification and serotyping

The MAPA-recommended isolation technique was used (SÃO PAULO, 1995). The drag swabs were homogenized, and 2 g of the material was inoculated in 20 mL of brain heart infusion (BHI) broth and incubated at 35 to 37 °C for 18 to 24 h (pre-enrichment). In the selective enrichment stage, the samples were homogenized, and 2 g of the material were inoculated into 20 mL of tetrathionate broth and 0.2 g into 20 mL of Rappaport-Vassiliadis broth and incubated at 42 to 43 °C for 18 to 24 h. In the isolation phase, Hektoen agar and bright green agar plates were striated from the pre-enrichment and selective enrichment broths and incubated at 35 to 37 °C for 18 to 24 h. After incubation, the colonies' appearance that had developed on the plates were observed (Hektoen agar showing blue-green colonies with or without a black center; and bright green agar showing pink colonies). To make a preliminary biochemical identification from agar, two to three colonies with *Salmonella* characteristics in the triple sugar iron (TSI), lysine iron (LIA), sulfide indole motility (SIM) and urea broth were picked from each plate. Strains that presented a biochemical profile compatible with *Salmonella* were antigenically characterized through the rapid agglutination test with somatic and flagellar antiserum. Considering that the poultry establishments in this study were unregistered, the legislation determines that all laboratory tests must be carried out in laboratories accredited to the national network of agricultural laboratories. In accordance with this legislation, epidemiological units were considered positive when at least one poultry shed had a positive diagnosis (BRAZIL, 2016).

Epidemiological data

Epidemiological information was collected by ADAPAR. The broilers were housed in screened commercial poultry sheds, but no information on lineage, age, sanitary status and vaccination was provided. The data collected were as follows: (1) type of exploitation (only broiler establishments were selected by the system); (2) poultry density (the system provided the number of birds housed in each epidemiological unit and in the surrounding units); and (3) presence of other animal species (pigs, dairy cattle, beef cattle, horses, dogs, fish, cats, sheep, goats, mules, birds reared for consumption by people at the establishment and donkeys) outside the commercial poultry sheds, in order to identify any associations between these factors and the presence of *S. enterica*. Birds reared for consumption by people at the establishment comprised those of low productivity that were kept free-range at the establishment and were unrelated to industrial production (these could be broilers or laying hens). The poultry density in the epidemiological unit was determined from the number of poultry sheds, housing capacity and average number of birds housed per poultry shed; and on other poultry farms in the area according to the number of poultry shed, breeding birds, hatching eggs, broilers and laying hens. Considering that most of the epidemiological units were located within a surrounding 5 km radius, this was adopted in other analyses of this study as a starting point.

Spatial analysis

In the ADAPAR system, the geographical coordinates of the epidemiological units were obtained from the point that formed the entrance to the establishment. Taking into account that the epidemiological units were considered positive when at least one poultry shed was positive, the official geographical coordinates of the establishment available in the system were used, rather than those of each poultry shed. This information was added to the database of the epidemiological variables, which made it possible to include this information in thematic maps. The maps were compiled using the ArcGIS software, version 10.5.1 (ESRI, 2017).

The analysis on distances had three aspects: distances between positive and negative epidemiological units; distances between epidemiological units and the town hall of the municipality and distances between epidemiological units and rivers and highways. For this, the means, minimums and maximums of the data were used, and these parameters were also subjected to statistical analysis for pairs of groups independently. In order to obtain the distances between epidemiological units, the nearest negative or positive point was considered, which also enabled the identification of transmissibility (positive to negative) and vulnerability (negative to positive) potentials.

The human development index (HDI) of the municipality was obtained (CHEDIEK et al., 2013). Environmental factors (temperature and precipitation) (AGRITEMPO, 2019) were evaluated in relation to the months in which samples for *Salmonella* investigation were collected. The average altitude of the regions studied (SILVEIRA, C.; SILVEIRA, R., 2017)

were obtained through the mean and standard deviation data from the municipalities. Statistical analysis was performed for pairs of independent groups.

Statistical analysis

A database was compiled from information collected through the ADAPAR system and through geoprocessing analyses. The variables analyzed were divided into two groups, as follows:

The first one is the associated factors: Presence/absence of other animals, i.e., other species present in the same epidemiological unit, but not necessarily in the commercial poultry sheds where the birds are housed. These animals circulate freely around the establishment, thus enabling dispersion of the agent, even though there is no real contact between commercial broilers and other species in the epidemiological unit. These animals may include pigs, dairy cattle, beef cattle, horses, dogs, fish, cats, sheep, goats, mules, birds (that are kept for consumption by people at the establishment) and donkeys.

Presence/absence of risk-posing establishments, i.e., establishments relating to rearing, slaughtering or industrial processing of birds and other animal species, along with small businesses that can carry the agent through vehicles and people transiting nearby, within the surrounding 5 km. These can include the following: farming stores (agricultural supply stores that can trade live birds), cattle slaughterhouses, poultry slaughterhouses, cold-storage warehouses (where meat is processed and stored after slaughter), agro-industrial cooperatives (free-membership organizations that contribute to their members' evolution in the economic and social spheres), fish producers and traders, dairies, animal protection societies, poultry integration companies (i.e., suppliers of one-day-old chicks, feed, technical assistance, medicines, housing support and air conditioning), supermarkets (which may contain products of animal origin without proper veterinary inspection) and smallholdings (small farms unrelated to cooperatives or poultry integration).

Poultry density variables, i.e., the total capacity of housing, number of poultry sheds and average number of birds housed per poultry shed in the epidemiological unit, and the poultry density in the surroundings. These were categorized and incorporated as categorical variables in the regression model. The data were categorized based on the most common categories of bird housing in the state of Paraná.

The prevalence ratio (PR) was used as a measurement of associations in bivariate analyses and the logistic regression model was used in multiple analyses. These multiple analyses with logistic regression models were performed with positive/negative epidemiological units as the dependent variable and the associated factors as independent variables. The method used to select models was stepwise. The model processing was started using a cutoff of $p < 0.20$ in bivariate analysis. Subsequently, to choose a better multiple model, p-values < 0.05 were used for each independent variable, r-square adjustments and interactions between independent variables as controls for confounding. The interpretation of the final models was based on the adjusted PR. The intensity of the association between the presence of disease and presence/absence of associated factors relating to presence of other animals, proximity to risk-posing establishments and poultry density was assessed using the PR and its corresponding confidence interval. The PR demonstrated the strength of association among poultry exposed to the study factor (disease) and how many times more likely it was that exposed birds would have the disease/condition than would unexposed birds (OLIVEIRA et al., 1997).

The second group is associated spatial variables: these comprised the distance between positive epidemiological units, distance between epidemiological units and highways, rivers and the town hall of the municipality (kilometers), municipal HDI (very low, low, medium, high or very high), mean monthly temperature (°C), the mean monthly precipitation (millimeters) and altitude (meters). In these analyses, the data were subjected to a normal distribution test. Student's t test was applied to parametric variables and the Mann–Whitney test to nonparametric variables. The results were interpreted considering a significance level of 5%. The data were also subjected to descriptive analysis to calculate means and frequencies.

The analyses were done with the aid of R environmental software (R CORE TEAM, 2019), through the "epitools" package (ARAGON, 2020), and IBM SPSS software, version 21 (IBM CORP, 2012).

RESULTS

General aspects

In Paraná, there are 399 municipalities with 10,804 epidemiological units and 18,974 poultry sheds, distributed across 81.20% of the state, including both the registered and unregistered units. In total, 243 unregistered epidemiological units

in 60 municipalities were analyzed, i.e., 2.25% of the total number of epidemiological units in the state. It was verified that, among the epidemiological units studied, 78 (32.10%) were positive. The map shown in Figure 1 highlights two regions in which positive cases were concentrated, in the northwest and west of the state.

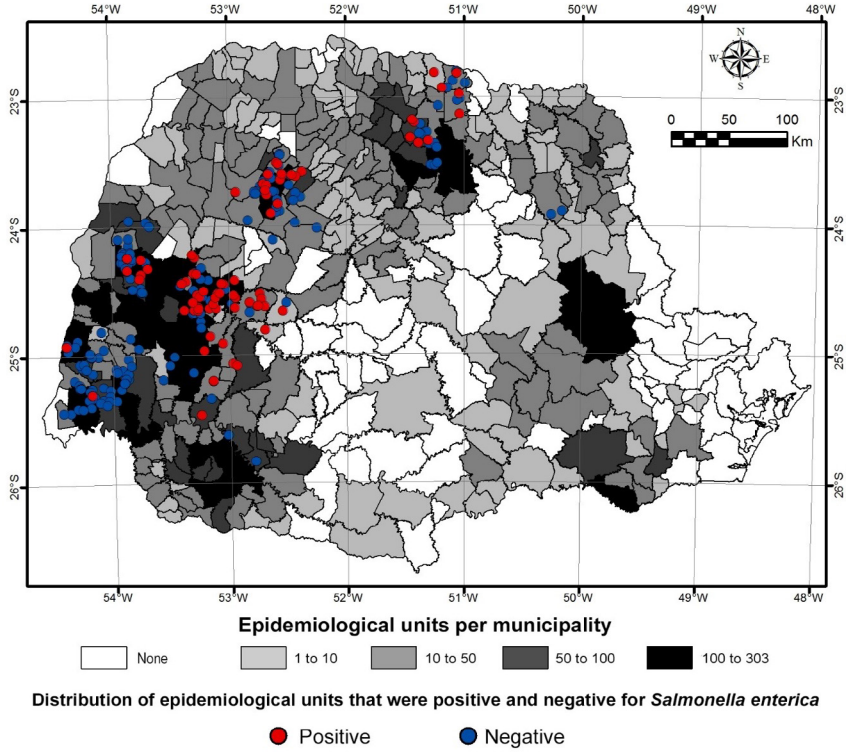

Figure 1. The distribution of epidemiological units that were positive and negative for *Salmonella enterica*, overlain on poultry density data for the state of Paraná.
Source: Elaborated by the authors.

Associated factor analysis for *Salmonella* infection: contact with other animals

In this study, among the epidemiological units at which other animals were present, 24.5% were positive and 75.5% negative. Among those at which no other animals were present, 38.3% were positive and 61.7% negative. Among the epidemiological units with the presence of other animals, a higher percentage was negative. Analysis on the prevalence ratios showed that there was a statistically significant protective association between *Salmonella* isolation and contact with other animals (prevalence ratio, PR = 0.64; 95% confidence interval, CI = 0.43–0.95; p = 0.022) (Table 1).

Table 1. The presence of other animals in positive and negative epidemiological units.

Exposure factor	Positive	Negative	Total	PR	P value
Contact with pigs					
Yes	10 (25.0%)	30 (75.0%)	40 (100.0%)	0.75 (0.42–1.32)	0.293
No	68 (33.5%)	135 (66.5%)	203 (100.0%)		
Contact with dairy cattle					
Yes	15 (24.6%)	46 (75.4%)	61 (100.0%)	0.71 (0.43–1.15)	0.147
No	63 (34.6%)	119 (65.4%)	182 (100.0%)		
Contact with beef cattle					
Yes	21 (31.8%)	45 (68.2%)	66 (100.0%)	0.98 (0.65–1.49)	0.954
No	57 (32.2%)	120 (67.8%)	177 (100.0%)		

continue...

Table 1. Continuation...

Exposure factor	Positive	Negative	Total	PR	P value
Contact with horses					
Yes	5 (23.8%)	16 (76.2%)	21 (100.0%)	0.72 (0.33–1.59)	0.395
No	73 (32.9%)	149 (67.1%)	222 (100.0%)		
Contact with dogs					
Yes	3 (20.0%)	12 (80.0%)	15 (100.0%)	0.61 (0.22–1.70)	0.300
No	75 (32.9%)	153 (67.1%)	228 (100.0%)		
Contact with fish					
Yes	2 (14.3%)	12 (85.7%)	14 (100.0%)	0.43 (0.12–1.57)	0.141
No	76 (33.2%)	153 (66.8%)	229 (100.0%)		
Contact with cats					
Yes	2 (25.0%)	6 (75.0%)	8 (100.0%)	0.77 (0.23–2.60)	0.661
No	76 (32.3%)	159 (67.7%)	235 (100.0%)		
Contact with sheep					
Yes	2 (25.0%)	6 (75.0%)	8 (100.0%)	0.77 (0.23–2.60)	0.662
No	76 (32.3%)	159 (67.7%)	235 (100.0%)		
Contact with goats					
Yes	2 (40.0%)	3 (60.0%)	5 (100.0%)	1.25 (0.42–3.72)	0.702
No	76 (31.9%)	162 (68.1%)	238 (100.0%)		
Contact with mules					
Yes	0 (0.0%)	1 (100.0%)	1 (100.0%)	*	0.490
No	78 (32.2%)	164 (67.8%)	242 (100.0%)		
Contact with birds for consumption by people at the unit					
Yes	0 (0.0%)	1 (100.0%)	1 (100.0%)	*	0.490
No	78 (32.25%)	164 (67.8%)	242 (100.0%)		
Contact with donkeys					
Yes	1 (100.0%)	0 (0.0%)	1 (100.0%)	3.14 (2.61–3.78)	0.145
No	77 (31.8%)	165 (68.2%)	242 (100.0%)		
Presence/absence of other animals					
Presence	27 (24.5%)	83 (75.5%)	110 (100.0%)	0.64 (0.43–0.95)	0.022
Absence	51 (38.3%)	82 (61.7%)	133 (100.0%)		

*Prevalence ratio could not be calculated.
Source: Elaborated by the authors.

Associated factor analysis for *Salmonella* infection: proximity of risk-posing establishments

In this study, among epidemiological units located within 5 km of risk-posing establishments, 20% were positive and 80% negative. Among those that were not close to these establishments, 39.2% were positive and 60.8% negative. Thus, a high percentage of epidemiological units near risk-posing places was negative and the prevalence ratio confirmed that there was a statistically significant protective association between *Salmonella* isolation and proximity to these sites (PR = 0.51; 95% CI = 0.32–0.81; p = 0.001). Similarly, there was a statistically significant association with the presence of agro-industrial cooperatives in the surroundings, but for protection (PR = 0.41; 95% CI = 0.18–0.93; p = 0.015) (Table 2).

Table 2. Proximity to risk-posing establishments within 5 km of epidemiological units that were positive and negative for *Salmonella enterica*.

Exposure factor	Positive	Negative	Total	PR	P value
Farming store					
Yes	12 (22.2%)	42 (77.8%)	54 (100.0%)	0.64 (0.37–1.09)	0.078
No	66 (3.9%)	123 (65.1%)	189 (100.0%)		
Cattle slaughterhouse					
Yes	1 (25.0%)	3 (75.0%)	4 (100.0%)	0.77 (0.14–4.28)	0.759
No	77 (32.2%)	162 (67.8%)	239 (100.0%)		
Poultry slaughterhouse					
Yes	2 (50.0%)	2 (50.0%)	4 (100.0%)	1.57 (0.58–4.26)	0.439
No	76 (31.8%)	163 (68.2%)	239 (100.0%)		
Cold-storage facility					
Yes	0 (0.0%)	15 (100.0%)	15 (100.0%)	*	0.006
No	78 (34.2%)	150 (65.8%)	228 (100.0%)		
Agro-industrial cooperative					
Yes	5 (14.3%)	30 (85.7%)	35 (100.0%)	0.41 (0.18–0.93)	0.015
No	73 (35.1%)	135 (64.9%)	208 (100.0%)		
Fish production or trading					
Yes	1 (20.0%)	4 (80.0%)	5 (100.0%)	0.62 (0.11–3.60)	0.558
No	77 (32.4%)	161 (67.6%)	238 (100.0%)		
Dairy					
Yes	7 (28.0%)	18 (72.0%)	25 (100.0%)	0.86 (0.44–1.66)	0.643
No	71 (32.6%)	147 (67.4%)	218 (100.0%)		
Animal protection society					
Yes	0 (0.0%)	1 (100.0%)	1 (100.0%)	*	0.491
No	78 (32.2%)	164 (67.8%)	242 (100.0%)		
Poultry integration company					
Yes	1 (16.7%)	5 (83.3%)	6 (100.0%)	0.51 (0.08–3.10)	0.412
No	77 (32.5%)	160 (67.5%)	237 (100.0%)		
Supermarket					
Yes	0 (0.0%)	5 (100.0%)	5 (100.0%)	*	0.120
No	78 (32.8%)	160 (67.2%)	238 (100.0%)		
Individual farm					
Yes	0 (0.0%)	1 (100.0%)	1 (100.0%)	*	0.491
No	78 (32.2%)	164 (67.8%)	242 (100.0%)		
Slaughterhouse and cold-storage facility					
Yes	1 (33.3%)	2 (66.7%)	3 (100.0%)	1.04 (0.21–5.20)	0.963
No	77 (32.1%)	163 (67.9%)	240 (100.0%)		
Presence/absence of risk-posing establishments					
Presence	18 (20.0%)	72 (80.0%)	90 (100.0%)	0.51 (0.32–0.81)	0.001
Absence	60 (39.2%)	93 (60.8%)	153 (100.0%)		

*Prevalence ratio could not be calculated.
Source: Elaborated by the authors.

Associated factor analysis for *Salmonella* infection: poultry density in the epidemiological unit

Table 3 shows the parameters of the number of poultry sheds, housing capacity and average number of birds per poultry shed for both positive and negative epidemiological units. The poultry density around the epidemiological units (poultry shed, breeding birds, hatching eggs, laying hens and broilers) was also analyzed.

Table 3. Poultry density within 5 km of epidemiological units that were positive and negative for *Salmonella enterica*.

Exposure factor	Positive	Negative	Total	PR	P value
Number of poultry sheds					
≤ 2	63 (31.0%)	140 (69.0%)	203 (100.0%)	Ref.	
3–5	14 (38.9%)	22 (61.1%)	36 (100.0%)	1.25 (0.79–1.98)	0.353
> 5	1 (25.0%)	3 (75.0%)	4 (100.0%)	0.80 (0.14–4.45)	0.796
Total housing capacity					
≤ 30,000	28 (25.0%)	84 (75.0%)	112 (100.0%)	Ref.	
30,001–150,000	49 (38.3%)	79 (61.7%)	128 (100.0%)	1.53 (1.04–2.26)	0.028
> 150,000	1 (33.3%)	2 (66.7%)	3 (100.0%)	1.33 (0.26–6.82)	0.742
Average number of birds per poultry shed					
≤ 30,500	54 (27.8%)	140 (72.2%)	194 (100.0%)	Ref.	
30,501–32,500	10 (71.4%)	4 (28.6%)	14 (100.0%)	2.57 (1.72–3.83)	0.001
32,501–37,000	14 (42.4%)	19 (57.6%)	33 (100.0%)	1.52 (0.96–2.41)	0.091
> 37,000	0 (00.0%)	2 (100.0%)	2 (100.0%)	*	0.381
Poultry sheds in surroundings					
≤ 50	55 (35.7%)	99 (64.3%)	154 (100.0%)	Ref.	
51–100	12 (18.5%)	53 (81.5%)	65 (100.0%)	0.52 (0.30–0.90)	0.011
> 100	11 (45.8%)	13 (54.2%)	24 (100.0%)	1.28 (0.79–2.08)	0.340
Breeding birds in surroundings					
≤ 50,000	63 (29.6%)	150 (70.4%)	213 (100.0%)	Ref.	
50,001–100,000	5 (45.5%)	6 (54.5%)	11 (100.0%)	1.54 (0.78–3.03)	0.264
> 100,000	10 (52.6%)	9 (47.4%)	19 (100.0%)	1.78 (1.11–2.86)	0.038
Hatching eggs in surroundings					
≤ 5,000,000	73 (31.5%)	159 (68.5%)	232 (100.0%)	Ref.	
5,000,001–10,000,000	4 (44.4%)	5 (55.6%)	9 (100.0%)	1.41 (0.66–3.00)	0.413
> 10,000,000	1 (100.0%)	1 (100.0%)	2 (100.0%)	1.59 (0.39–6.44)	0.575
Broilers in surroundings					
≤ 500,000	27 (36.5%)	47 (63.5%)	74 (100.0%)	Ref.	
500,001–1,000,000	21 (29.2%)	51 (70.8%)	72 (100.0%)	0.80 (0.50–1.28)	0.346
> 1,000,000	30 (30.9%)	67 (69.1%)	97 (100.0%)	0.85 (0.55–1.29)	0.445
Laying hens in surroundings					
≤ 50,000	75 (34.1%)	145 (65.9%)	220 (100.0%)	Ref.	
50,001–100,000	0 (00.0%)	9 (100.0%)	9 (100.0%)	*	0.033
> 100,000	3 (21.4%)	11 (78.6%)	14 (100.0%)	0.63 (0.23–1.74)	0.330

"Ref." means that the next values were calculated based on this number; * means that no calculation was possible.
Source: Elaborated by the authors.

In both the *S. enterica*-positive and the negative epidemiological units, up to two poultry sheds were predominant per epidemiological unit. The total housing capacity in the negative epidemiological units was higher than that of the positive units. There was a statistically significant association between the risk of *Salmonella* isolation and the average housing capacity of 30,001 to 150,000 (PR = 1.53; 95% CI = 1.04-2.26; p = 0.028). The average number of birds per poultry shed was higher in positive epidemiological units and, through analyzing the prevalence ratio, it was confirmed that there was a statistically significant association between the risk of *Salmonella* isolation and an average number of birds per shed of 30,501–32,500 (PR = 2.57; 95% CI = 1.72–3.83; p = 0.001). This suggests that this number of birds housed per poultry shed may increase the incidence of *S. enterica*.

Regarding the parameter of surrounding poultry sheds, the highest proportion was around negative epidemiological units. The category 51–100 showed a statistically significant association with protection (PR = 0.52; 95% CI = 0.30–0.90; p = 0.011). In the analysis of breeding birds surrounding the epidemiological unit, there was a subtle difference between positive (relatively higher) and negative in the > 100,000 category. Analysis of the prevalence ratio confirmed that there was a statistically significant association between the risk of isolation of *Salmonella* and this category (PR = 1.78; 95% CI = 1.11–2.86; p = 0.038). The proportion of hatching eggs in the surroundings was higher in negative epidemiological units, especially in the category ≤ 5,000,000, as also was the proportion of broilers in the category > 1,000,000. For laying hens in the surroundings, there was a statistically significant association in the 50,001–100,000 category because there was no positive count and therefore, it was not possible to calculate the prevalence ratio.

Logistic regression analysis

Through logistic regression analysis for *Salmonella*, it was found that the largest associations were related to the average number of birds per poultry shed (30,501 to 32,500 [ref. ≤ 30,500]), the surrounding establishments that posed a risk (yes [ref. no.]); and the number of surrounding poultry sheds (over 5 [ref. 0 to 2]). Considering all the variables together (contact with other animals, proximity to risk-posing establishments and poultry density), it could be seen that these factors produced a significant multiple model for *Salmonella* (Table 4), except for the parameter of surrounding risk-posing establishments, which remained protective even after controlling for interactions. Other binary logistic regression models were tested using variables with p-values ≤ 0.20 in the bivariate analysis. However, these variables were excluded from the model because they did not show any p values < 0.05.

Table 4. Multiple logistic regression model using the significant variables

Significant variables*	Adjusted PR (95% CI)	P value
Average number of birds per poultry shed: 30,501 to 32,500 (ref. ≤ 30,500)	6.93 (1.02–23.81)	0.002
Risk-posing establishments in surroundings: yes (ref. no.)	0.42 (0.22–0.79)	0.007
Poultry sheds in surroundings: more than 5 (ref. 0 to 2)	2.79 (1.04–7.47)	0.041

*Controlled for interactions between independent variables.
Source: Elaborated by the authors.

Spatial analysis

Distance analysis between epidemiological units

Table 5 shows the distances in kilometers (km) from positive epidemiological units to the nearest other positive unit, from positive units to the nearest negative unit and from negative units to the nearest positive unit.

In general, the smallest distance between a positive epidemiological unit and the nearest other positive one was 100 m. Analysis of the distances between positive establishments and the nearest negative ones showed that the minimum was 130 m and the maximum was 36.35 km. Thus, there was a potential for transmission from positive to negative units over small distances. Analysis of the distances from negative to positive units showed the potential vulnerability, considering that the smaller the distance to a positive epidemiological unit was, the more vulnerable to infection a negative epidemiological unit became. However, in this context, the range between the minimum and maximum distance was considerable, since the shortest distance was 130 m and the longest was 121.92 km.

It was noticed that positive epidemiological units showed lower means and ranges of distances than negative units. This allows the hypothesis that the positive units had greater potential for transmission of *S. enterica*. This parameter presented a statistical difference according to the Mann–Whitney test (p < 0.05).

Considering only the positive epidemiological units, most of them (58.97%) were within 5 km of other positive ones, while 34.62% were between 5 and 20 km away from each other (Fig. 2).

Table 5. General distances (km) between the epidemiological units studied and distance analysis between positive and negative epidemiological units and between these and the town hall of the municipality, highways and rivers.

Distance to nearest epidemiological unit	Minimum	Maximum	Mean	P value
Positive to positive	0.10	47.39	7.35	0
Positive to negative	0.13	36.35	6.83	
Negative to positive	0.13	121.92	14.84	

Other distances	Positive			Negative			P value
	Mean	Median	SD	Mean	Median	SD	
Town hall of the municipality	8.96	8.50	4.97	8.67	8.66	4.29	0.639*
Highways	3.17	2.57	2.74	2.68	2.14	2.23	0.282
Rivers	0.27	0.20	0.21	0.23	0.18	0.18	0.335

*Student's t test.
Source: Elaborated by the authors.

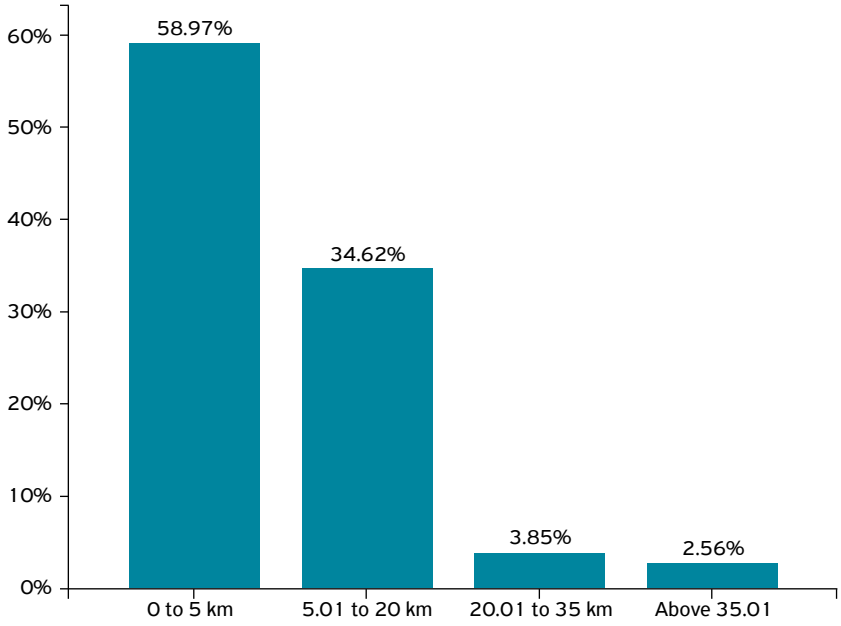

Figure 2. Distances between positive epidemiological units.
Source: Elaborated by the authors.

Distance analysis between epidemiological units, town hall of the municipality, highways and rivers

Table 5 presents distance data between the positive epidemiological units and the town hall of the municipality, highways and rivers. From this, it can be seen that there were no statistical differences regarding these parameters. This suggesting that they did not have any influence on the presence of *S. enterica* in this study.

Analysis of the municipal HDI among the municipalities of the epidemiological units studied

Figure 3 shows the HDI of the municipalities studied (CHEDIEK et al., 2013), with emphasis on positive and negative cases. Among these municipalities, the HDI was classified as high in 31% of the positive cases, while it was high in 69% of the negative cases. Among municipalities with average HDI, 40.7% were positive and 59.3% were negative. There was no statistical difference in this parameter ($p > 0.05$), while the prevalence ratio indicated that high HDI was a protective factor among epidemiological units exposed to the disease (Table 6).

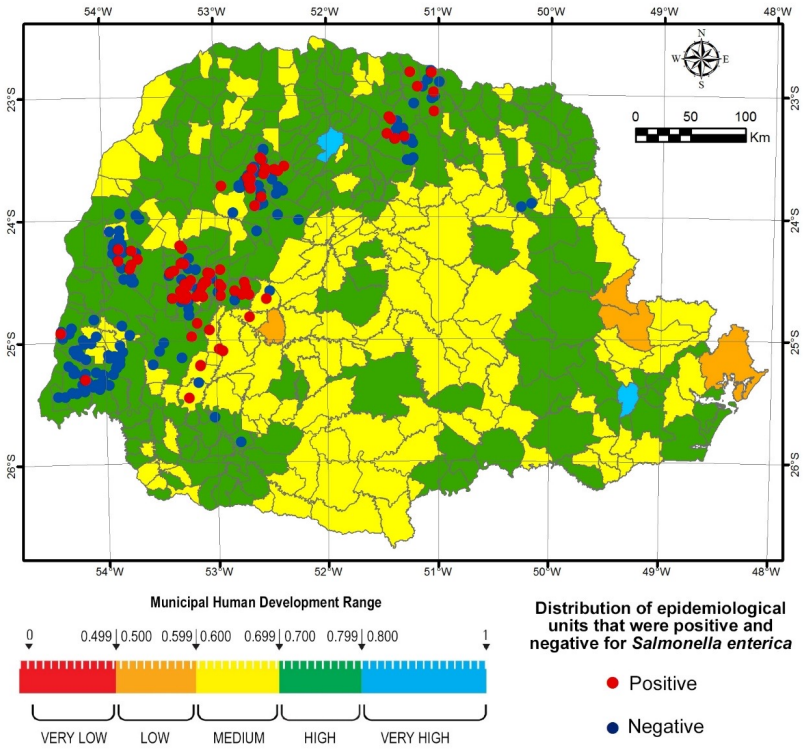

Figure 3. The municipal human development index (HDI) distribution in the state of Paraná and epidemiological units that were positive and negative for *Salmonella enterica*.
Source: Elaborated by the authors.

Table 6. The municipal human development index (HDI) in municipalities with epidemiological units that were positive and negative for *Salmonella enterica*.

Municipal HDI	Positive	Negative	Total	PR	P value
Medium	11 (40.7%)	16 (59.3%)	27 (100.0%)	0.76 (0.46–1.25)	0.308
High	67 (31.0%)	149 (69.0%)	216 (100.0%)		

Source: Elaborated by the authors.

Temperature (°C) in the municipalities of the epidemiological units studied

Table 7 shows the average monthly temperatures in the municipalities studied (AGRITEMPO, 2019), especially in the positive and negative cases. This parameter showed little variation between positive and negative cases and there was no statistical difference according to the Mann–Whitney test. This suggests that this variable was not related to *S. enterica* infection in this analysis.

Table 7. The temperature, precipitation and altitude distribution in the state of Paraná and epidemiological units that were positive and negative for *Salmonella enterica*.

	Positive			Negative			p value
	Mean	Median	SD	Mean	Median	SD	
Temperature	25.14	25.13	1.51	25.30	25.23	1.44	0.593
Precipitation	5.63	5.30	2.53	5.83	5.63	2.37	0.265
Altitude	478.26	460.99	119.88	434.98	426.32	133.18	0.023

Source: Elaborated by the authors.

Precipitation (mm) in the municipalities of the epidemiological units studied

Table 7 shows the precipitation in the municipalities studied (AGRITEMPO, 2019), with emphasis on positive and negative cases. This parameter showed little variation between positive and negative cases and there was no statistical difference according to the Mann–Whitney test. This suggests that this variable was not related to *S. enterica* infection in this study.

Altitude (m) in the municipalities of the epidemiological units studied

Figure 4 and Table 7 demonstrate the altitudes of the municipalities studied (SILVEIRA, C.; SILVEIRA, R., 2017), with emphasis on positive and negative cases. In this study, it was observed that the municipalities of the epidemiological units that were positive for *S. enterica* were at higher altitudes than those with negative epidemiological units ($p < 0.05$).

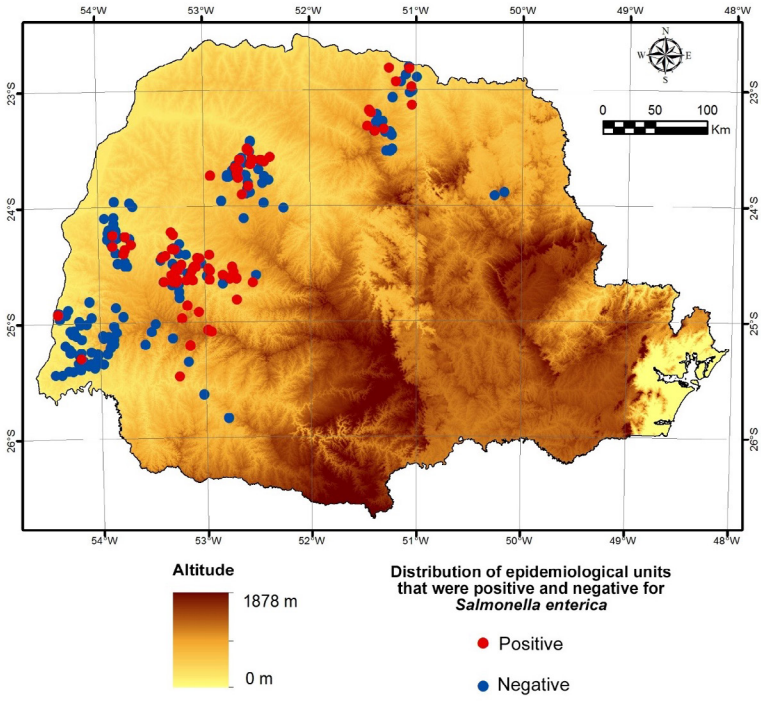

Figure 4. The altitude distribution in the state of Paraná and epidemiological units that were positive and negative for *S. enterica*. Source: Elaborated by the authors.

DISCUSSION

Was identified 78 positive epidemiological units among the 243 epidemiological units studied. The positivity rate was, therefore, 32.10%. In another study conducted in Paraná (PANDINI et al., 2015), of 342 drag swab samples that came from epidemiological units in this state, 11.4% were positive for *S. enterica*. This difference in positivity can be attributed to a more careful analysis, after implementation of Normative Instruction No. 20 of October 21, 2016 (BRAZIL, 2016). The epidemiological units studied were located in municipalities predominantly in the northern, northwestern, western, southwestern and northeastern regions of Paraná (Fig. 1). It could be seen that the northwestern and western regions had higher concentrations of positive cases than the other regions studied.

The presence of animals other than poultry in poultry production establishments may also be a factor associated with *S. enterica* infection (ELGROUD et al., 2009) in broilers. This is because, among other factors, the traffic of people in one way or another can carry the bacteria, thus favoring dissemination of the agent in the establishment (LE BOUQUIN et al., 2010). This study demonstrated that a higher percentage of epidemiological units with the presence of other animals were negative. However, as previously reported in other studies (BOUWKNEGT et al., 2004; ELGROUD et al., 2009), it was expected that establishments with the presence of other animals would be more predisposed to positivity for pathogenic bacteria than those that did not have any other animals on the establishment. The low competitiveness of *Salmonella* in relation to other microorganisms may be an explanation for these findings (AHO, 1992; BARROW, 2000), which may

therefore result in low prevalence of infection in the batch (ARNOLD et al., 2005). In addition, the birds only release *S. enterica* intermittently, in small quantities in their feces, which makes it difficult to detect the bacteria (BOUWKNEGT et al., 2004). From another perspective, bacterial resistance varies under adverse environmental conditions, and this may hinder isolation of *S. enterica*, especially with regard to assessing epidemiological units that present low biosafety, as was the case in this study. This is due to the large number of other species of bacteria in the environment (mainly lactic acid), against which *Salmonella* has low competitive capacity (BONI et al., 2011).

The proximity of companies or establishments that own animals or handle animal products unregulated can be considered to be a risk to poultry production because this influences transmission of *S. enterica* to the epidemiological units through the transit of animals and people. A study developed in France showed that the risk of *Salmonella* contamination on farms increased when feed trucks were parked near the entrance to the poultry sheds. It was shown that contamination of vehicles and footwear was a source of *Salmonella* infection in poultry farms. Thus, mechanical dissemination of microorganisms by vehicles and on the footwear of people who are traveling in the vicinity can give rise to a risk of *Salmonella* positivity in broilers (ROSE et al., 1999). However, in this study, most of the nearby epidemiological units were negative, thus suggesting that this parameter did not have any influence. Although these establishments were not evaluated for the presence of *Salmonella*, it was expected that proximity to them would favor infection, considering the flow of animals and people from places with questionable practices around the epidemiological units studied.

One of the main risk factors associated with *S. enterica* infection in poultry farms is the poultry density (ELGROUD et al., 2009), since the excretion of the bacteria by affected birds can infect the whole batch and even nearby batches without any presence of apparent clinical signs (PEREIRA et al., 1999). Among laying hens, batch size was also considered to be a major risk factor for *S. enterica* infection (HUNEAU-SALAÜN et al., 2009).

In the bivariate analysis of factors associated with *Salmonella*, the variables with significant p-values were the following: (1) presence or absence of other animals, contact with dairy cattle, contact with fish and contact with donkeys; (2) presence of agricultural stores, cold-storage facilities, agro-industrial cooperatives, risk-posing establishments and supermarkets; and (3) total housing capacity, average number of birds per poultry shed, number of surrounding poultry sheds, presence of breeding birds and presence of laying hens. Some logistic regression models were obtained from these variables, but in the final model only the average number of birds per poultry shed (30,501 to 32,500 [ref. ≤ 30,500]), surrounding the presence of risk-posing establishments (yes [ref. no.]) and number of surrounding poultry sheds (above 5 [ref. 0 to 2]) were retained. This information can be considered to form a part of the *Salmonella* control strategy, through epidemiological surveillance inside and around the poultry sheds.

Geoprocessing has been used in epidemiological studies for spatial localization of disease cases, environmental correlations and analysis on distance variables, with the aim of obtaining information about and controlling these diseases (LÚCIO et al., 2018). In the distance analysis among the epidemiological units studied, it was noticed that the positive epidemiological units had lower means and ranges of distances than the negative units, thus allowing the hypothesis that they had greater potential for transmission of *S. enterica*. This parameter presented statistical difference according to the Mann–Whitney test ($p < 0.05$) (Table 5). Considering only the positive epidemiological units, most of them were within 5 km of other positive ones. Geographical proximity between epidemiological units may allow a hypothesis of favored dissemination of the agent (NAMATA et al., 2009), which could occur through the air or by means of vehicles, vectors, people, etc. In this light, it would be appropriate for new epidemiological units to be constructed at distances of more than 5 km from each other. The distance to the nearest epidemiological unit is a factor that can interact with the poultry density, because the greater the proximity is, the greater the size of the population at risk is (SNOW et al., 2010). In this study, it was observed that the positive epidemiological units were close to the negative ones, which suggested that the potential for transmission of *S. enterica* existed. Proximity to the town hall of the municipality and to highways might indicate greater flow of vehicles and proximity to rivers might indicate agglomerations of other animal species, especially wild birds, which could represent risk factors for *S. enterica* infection in broilers. Nonetheless, it was found that there were no statistical differences in relation to these parameters, thus suggesting that they did not have any influence on the presence of *S. enterica* in this study.

The HDI measures the living conditions of a population in terms of social and economic aspects. It was created by the United Nations Development Program (UNDP) in the 1980s. This index encompasses factors such as health, education and income. The municipal HDI measures the same parameters, but reflect the particularities of each municipality. It can range from zero to one, such that the level of human development is greater when the index is closer to one (CHEDIEK et al., 2013). The evaluation among the municipalities studied showed that the positive and negative epidemiological units were located predominantly in municipalities with high HDI. Worse HDI conditions could be expected to influence

aspects of the education of people involved in poultry work and thus could favor *Salmonella* infection in epidemiological units through inappropriate management practices, for example.

Environmental factors are closely related to the results obtained from poultry production. In situations of temperatures lower than recommended, poultry initially reduce their food consumption and energy mobilization reserves for thermogenesis, which negatively influences their performance. On the other hand, increasing ambient temperatures due to climatic variations and use of different types of poultry shed are one of the most important issues in Brazilian poultry productivity. This may alter the functioning of the immune system, decrease resistance to infections and impair bird performance (QUINTEIRO-FILHO et al., 2010). In these situations, several negative physiological effects are observed, especially in the immune system, thus increasing the susceptibility to opportunistic diseases.

The combination of caloric stress and *S.* Enteritidis infection can disrupt the intestinal barrier of poultry. This allows the migration of bacteria through the intestinal mucosa to other organs and can also cause intestinal inflammation, thus reducing performance parameters (QUINTEIRO-FILHO et al., 2012). In this study, the temperature parameter showed little variation between positive and negative, and there was no statistical difference ($p > 0.05$) according to the Mann–Whitney test (Table 7). This suggests that this variable was not related to *S. enterica* infection in the present analysis. Although no statistical differences regarding the temperatures in the municipalities were observed in this study, it is valid to consider that oscillations can occur during the year. However, this did not necessarily influence the average values used in this analysis.

It has been emphasized that studying climatic variables is important, especially within the field of bioclimatology (SANTOS et al., 2014). For production birds, it is possible to establish the geographical areas that are favorable for them, and those in which structural adjustments are required in order to provide thermal comfort for them. This is enabled through knowledge of the environmental needs of birds and the climatic conditions of the region. In a study evaluating risk factors for *Campylobacter* infection, it was found that high frequencies and volumes of the rain associated with temperature elevation increased the possibility of occurrence of this microorganism in broilers (JONSSON et al., 2012). However, the precipitation parameter in this study, as shown in Table 7, did not any present statistical difference according to the Mann–Whitney test ($p > 0.05$), thus suggesting that it did not have any relationship with *S. enterica* infection.

The altitude can influence the air temperature and this relationship is especially important in tropical and subtropical regions, where altitude differences cause changes in climate, soil, vegetation and, consequently, the plants and animals' physiological processes (FRITZSONS et al., 2016). High altitudes have been shown to make temperature changes problematic because according to the characteristics of the location, increases in relative air humidity indexes may adversely affect exchanges and result in higher values for comfort parameters, like the temperature and humidity index (ITU) (KARKOW, 2015). Results from a meta-analysis showed that higher altitude is related to lower weight gain and higher mortality among broilers, regardless of sex and age at slaughter. In this study, it was observed that the municipalities with epidemiological units that were positive for *S. enterica* were at higher altitudes than those with negative epidemiological units ($p < 0.05$).

It was found through this study that positivity for *S. enterica* in the epidemiological units studied was unrelated to the presence of animal species other than poultry. Moreover, most positive units did not have any presence of other animals. This can be attributed to the low competitiveness of *Salmonella* in the presence of other microorganisms, which prevented isolation. Alternatively, there may really has been no causal relationship. It was also observed that higher poultry density in the facilities may influence positivity for *Salmonella*. However, the poultry density in the surroundings did not present any statistical difference between positive and negative epidemiological units. Visually, the regions with the highest concentration of positive cases were located in the northwestern and western regions of the state. It was found that high altitude appeared to influence *S. enterica* positivity.

Based on the results reported in this paper, it is important to draw attention to some limitations to this study regarding *Salmonella* control. There were difficulties at the time of data collection for this study, especially regarding changes to the registration information among producers, which changed through transfers of epidemiological units between family members. There was also an absence of animal transit guide (ATG) information, which would have enabled analysis on the length of time for which sheds remained empty for sanitation measures between batches, to determine whether there might have been any relationship between this parameter and *Salmonella* positivity. Data regarding the age of the broilers at the time of collection of drag swabs and data on their vaccination status could also have provided information about the particularities of isolation and the serovars involved. Information regarding the general handling measures used in these epidemiological units would have also contributed to the results from this study. Such information could be obtained if there were a biosafety checklist, thus allowing classification and profiling of these establishments. The lack of serotyping of all results was also a limitation because if all the results had indicated the serovars involved it would have been possible to ascertain the characteristics and particularities of these serovars.

It can be expected that in registered establishments, there will be a lower likelihood of *S. enterica* infection, considering all the biosafety measures that are adopted and the greater epidemiological surveillance that is implemented in these places than in unregistered establishments. As previously stated, the data made available for this study referred only to epidemiological units that were not registered at the time of sample collection. This is a limitation that prevented comparison between registered and unregistered epidemiological units. It is worth mentioning that the Brazilian government has encouraged the registration of commercial establishments through Normative Instruction No. 8 of February 17, 2017, which determined that commercial poultry establishments would be under an obligation to obtain registration within 365 days, among other measures (BRAZIL, 2017).

CONCLUSIONS

Considering that *Salmonella* serovars prevalence varies depending on the geographic location and time, factors related to the introduction and dissemination of these agents in poultry sheds are important for the adoption of effective control measures.

Based on the results of this study, it was found that a higher density broiler in poultry sheds, presence of surrounding poultry sheds, proximity between positive and negative epidemiological units and altitude of the municipality may be related to presence for *S. enterica*.

The information obtained in this study points to some factors related to positivity for *S. enterica* and reinforces the importance of serotyping to obtain other epidemiological data.

AUTHORS' CONTRIBUTIONS

Conceptualization: Santin, E. **Data curation:** Silva, N. D.; Laurindo, E. E. **Formal analysis:** Silva, N. D.; Laurindo, E. E.; Martins, C. M. **Investigation:** Silva, N. D.; Martins, C. M.; Silveira, R. M.; Silveira, C. T. **Validation:** Santin, E. **Writing – original draft:** Silva, N. D.; Santin, E. **Writing – review & editing:** Silva, N. D.; Santin, E.

AVAILABILITY OF DATA AND MATERIAL

Not applicable.

FUNDING

There was no source of funding for this study.

CONFLICTS OF INTEREST

The authors declare that this study was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

ETHICAL APPROVAL

Not applicable.

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